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UNIVERSITY OF ALBERTA

Syntheses of β-Lactone Antibiotics and their Analogs

by

Yunlong Pu ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Department of Chemistry

EDMONTON, ALBERTA

Fall 1992

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Syntheses of β-Lactone Antibiotics and their Analogs by Yunlong Pu in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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To my parents and Linuo

Abstract

A process has been developed in which *N*-(*o*-nitrophenyl)sulfenyl-protected L-threonine **21** is cyclized in one step via carboxyl group activation using 4-bromobenzenesulfonyl chloride in pyridine to the corresponding *N*-(*o*-nitrophenyl)sulfenyl L-threonine β-lactone (**23**) (45-56%). This can be subsequently deprotected by thiolysis (≥85%) and then acylated with a variety of reagents (80-92%). The β-lactone antibiotic SQ 26,517 (**1**) was synthesized using this approach in 28% total yield over 4 steps from L-threonine. Three analogs of **1** bearing a benzoyl (**38**), and (L)- or (D)-*N*-Boc-phenylalaninyl groups (**39** and **40**) on nitrogen were similarly prepared. Most nucleophiles (e.g., pyrazole or EtMgCl with $CuBr{\cdot}SMe_2$) do not react with the threonine β-lactone derivatives at the β-position; however, β-halo (Br or Cl) α-amino acids can be generated in good to excellent yields (68-100%) from either **23** (or L-*allo*-threonine β-lactone **32**) or **33** by treatment with the concentrated hydrogen halide solutions (either aqueous or in acetic acid).

Optically pure (+)-obafluorin (**2**), a novel β-lactone antibiotic, is synthesized in 7% total yield over seven steps through a key intermediate **6**, (2*S*,3*R*)-2-amino-3-hydroxy-4-(*p*-nitrophenyl)butanoic acid. This β-hydroxy α-amino acid is prepared stereospecifically by an aldol condensation between the enolate of (2*S*)-1-benzoyl-2-(*tert*-butyl)-3-methyl-4-imidazolidinone (**14**) and (*p*-nitrophenyl)acetaldehyde (**53**) followed by acidic hydrolysis of the aldol adduct. The syntheses of the β-lactone derivatives from amino acid **6** employed the same strategy as that used for the synthesis of SQ 26,517. *N*-Acetyl (**65**), *N*-benzoyl (**66**), and *N*-[2-(2-aminothiazol-4-yl)-2-(methoxy-imino)]acetyl (ATMO) (**67**) analogs of obafluorin (**2**) were similarly prepared. Three β-lactone tosylate salts bearing a *para*-substituted [X = H (**68**), Cl (**69**), or MeO (**70**)] benzyl group at the β-position were also synthesized to study the function of the aromatic nitro substitutent in **2**.

Based on the two presumed functions of the 2,3-dihydroxybenzoyl group in obafluorin (**2**), i.e., iron-chelating and/or hydrophilicity-promoting, eight acyl groups, such as tribenzyl EDTA, (bipyridyl)carbonyl, and L-α-aminoadipyl groups, were chosen to couple to the β-lactone nucleus. The syntheses of the acyl groups as the corresponding acids and their attachment to the L-threonine β-lactone tosylate salt **33** employing either a mixed anhydride approach or a peptide-coupling (DEPC and Et_3N) strategy are described. Nucleophilic substitution of bromoacetyl L-threonine β-lactone (**118**) with 6-mercatopurine or 4-pyridylsulfide gives the corresponding β-lactone derivatives.

Obafluorin (**2**) exhibited good antibacterial activity against *Staphylococcus aureus* strains, but its three analogs **65-67** bearing different acyl groups are devoid of activity. Several synthetic *N*-acyl threonine β-lactones showed better biological activity over a broader spectrum than the natural antibiotic SQ 26,517 (**1**). The *N*-(*o*-nitrophenyl)sulfenyl-protected β-lactones are the most potent compounds among all the β-lactone derivatives; more work is in progress to investigate their biological activity.

Acknowledgements

I am most grateful to my supervisor, Dr. John C. Vederas, for his excellent guidance, support, and encouragement during my studies. I would like to specially thank Dr. Chris Lowe for his collaborative work, helpful discussions, and proof-reading this manuscript. I also thank Dr. Fionna M. Martin for the collaborative work. Dr. Miloslav Sailer is gratefully acknowledged for his assistance with biological assays. I am indebted to Dr. Yuko Yoshizawa for proof-reading this manuscript. I thank all the members in our group for their helpful discussions: especially Dr. Yonghong Song, Mr William Sherwin and Mr Lei Qiao. The staff in spectral analytical services in the Department of Chemistry is thanked for their assistance in characterizing compounds. Finally, financial support from the University of Alberta is greatly appreciated.

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List of Abbreviations

Ac	acetyl
aq	aqueous
Ar	aromatic
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bu	butyl
*i*Bu	isobutyl
*t*Bu	*tert*-butyl
cat.	catalytic
Cbz	benzyloxycarbonyl
CI	chemical ionization
DCC	*N*, *N*'-dicyclohexylcarbodiimide
d.e.	diastereomeric excess
DEPC	diethylphosphoryl cyanide
DIBAL	diisobutylaluminum hydride
DMAD	dimethyl azodicarboxylate
DMAP	*N*, *N*-dimethylaminopyridine
DMF	*N*, *N*-dimethylformamide
DMP	2,2-dimethoxypropane
DMSO	dimethylsulfoxide
EDTA	ethylenediaminetetraacetic acid
Et	ethyl
FAB	fast-atom bombandment
HPLC	high performance liquid chromatography

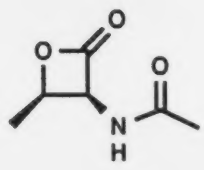
IR	infrared spectroscopy
LDA	lithium diisopropylamide
Me	methyl
MS	mass spectroscopy
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
Ph	phenyl
*n*Pr	propyl
iPr	isopropyl
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
Ts	*p*-toluenesulfonyl

Introduction

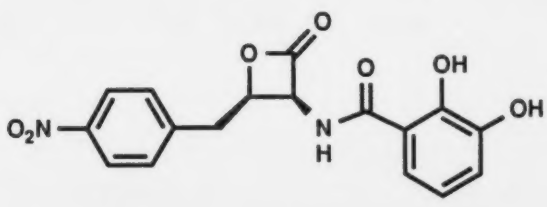
1. Background

For decades, β-lactam antibiotics (e.g., penicillins, cephalosporins, and other new types of β-lactams) have been the primary agents used to combat bacterial infections;[1-4] however, there is a continuing need for new drugs to attack bacterial strains that have developed resistance to the traditional treatments. In the last ten years, a number of α-amino β-lactone antibiotics have been isolated as microbial metabolites

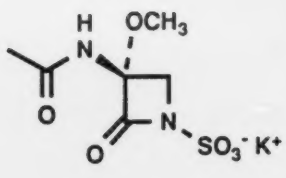
Figure 1. Examples of naturally occurring β-lactone and β-lactam antibiotics.



1 SQ 26,517



2 (+)-Obafluorin



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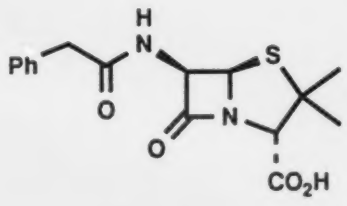


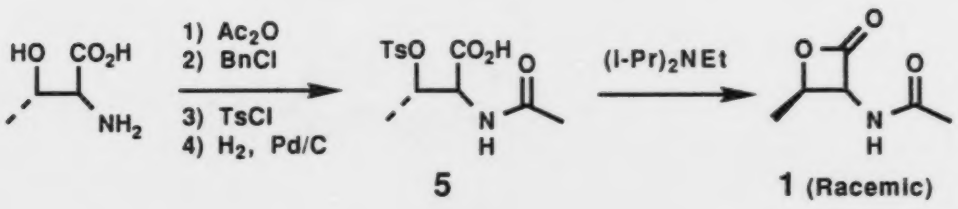
4 Penicillin G

during the screening for new β-lactam antibiotics.[5-12] These compounds are exemplified by SQ 26,517 (**1**)[5,6] and obafluorin (**2**).[7-9] The β-lactone functionality is rarely encountered in secondary metabolites,[13] but, when present, it is usually

associated with biological activity.[7,8,14,15] It is interesting to note that the structures of **1** and **2** (Figure 1) resemble those of the monobactam class of β-lactam antibiotics (e.g., **3**), and that they have stereochemistry associated with the β-lactone ring analogous to that of the β-lactam rings of penicillins (e.g., **4**) and cephalosporins.[8,9] A detailed X-ray study of obafluorin (**2**) and several similarly substituted monobactams suggests that β-lactones and β-lactams may be conformationally isosteric since their crystal structures reveal virtually identical ring conformations.[8] The mode of action of β-lactone antibiotics is still unknown, although it has been suggested that the biological activity appears to be a consequence of the β-lactone ring.[8]

SQ 26,517 (**1**), a β-lactone derived from L-threonine, was isolated from *Bacillus sp.* SC 11,480. This antibiotic displays weak antimicrobial activity against a number of strains.[5,6] Despite the simplicity of its structure, previous syntheses of this molecule have proceeded in very low yields.[6] Direct cyclization of *N*-acetyl threonine

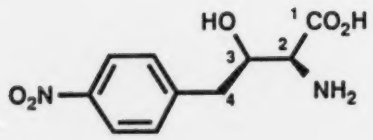
Figure 2. Synthesis of racemic SQ 26,517.



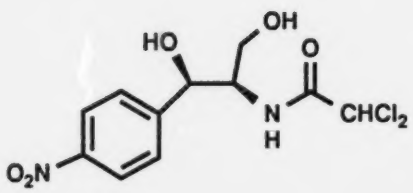
with DCC and DMAP produced the corresponding β-lactone in only 0.8% estimated yield.[6] An alternative method (Figure 2) involving activation of the hydroxyl group in *N*-acetyl-DL-*allo*-threonine to form the tosylate **5** provided racemic **1** in 1.6% yield over five steps.[6]

(+)-Obafluorin (**2**), a novel β-lactone antibiotic elaborated by *Pseudomonas fluorescens* (ATCC 39502), possesses unprecedented, albeit moderate, biological activity.[7-9] Studies on the interaction of obafluorin with β-lactamases showed that obafluorin was efficiently hydrolyzed by the enzymes tested.[7] This is the first β-lactone substrate for β-lactamases, and the first non-β-lactam antibiotic showing a high degree of susceptibility to hydrolysis by such proteins.[7,8] This observation and other biological investigations suggest that obafluorin acts in a specific manner reminiscent of β-lactam antibiotics, rather than as a general acylating agent.[8]

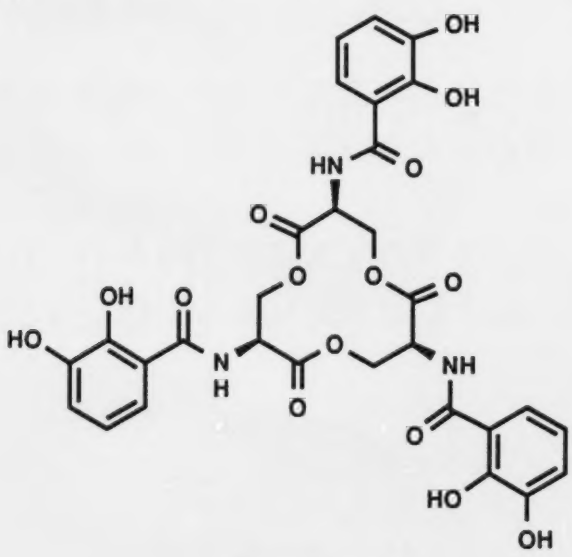
Several unusual functionalities reside in the uncomplicated structure of obafluorin. Both the central β-lactone ring and the aromatic nitro group are rare among natural products and are normally associated with biological activity.[7-9,13-15] The structure of the amino acid **6**, (*R*)-3-(*p*-nitrobenzyl)-L-serine, the key intermediate of obafluorin biosynthesis,[9] resembles that of chloramphenicol (**7**), which is produced by *Streptomyces venezuelae* and is used for treatment of typhoid infections.[16-18] Compound **6** contains a unique four carbon amino acid unit attached to the aromatic ring in contrast to the three carbon unit present in common aromatic amino acids such as phenylalanine. The 2,3-dihydroxybenzamide moiety also occurs naturally in certain microbial siderophores.[8] Siderophores are the iron-binding molecules secreted from the iron acquisition system evolved by bacteria to acquire essential iron from the environment,[19-21] and are recognized by specific receptors on their outer membranes. The most powerful natural siderophore is enterobactin (**8**), a cyclic trimer of *N*-(2,3-dihydroxybenzoyl)-L-serine, with a binding constant for ferric ion of 10^{49}.[19-21] Notably, several pharmaceutical companies have reported that penicillins, cephalosporins and monobactams bearing catechol or other iron chelating groups have shown much enhanced activity against certain Gram-negative bacteria; this may be due to improved penetration of the drugs through the outer bacterial membrane.[22-27]



6 3(*R*)-(*p*-Nitrobenzyl)-L-serine



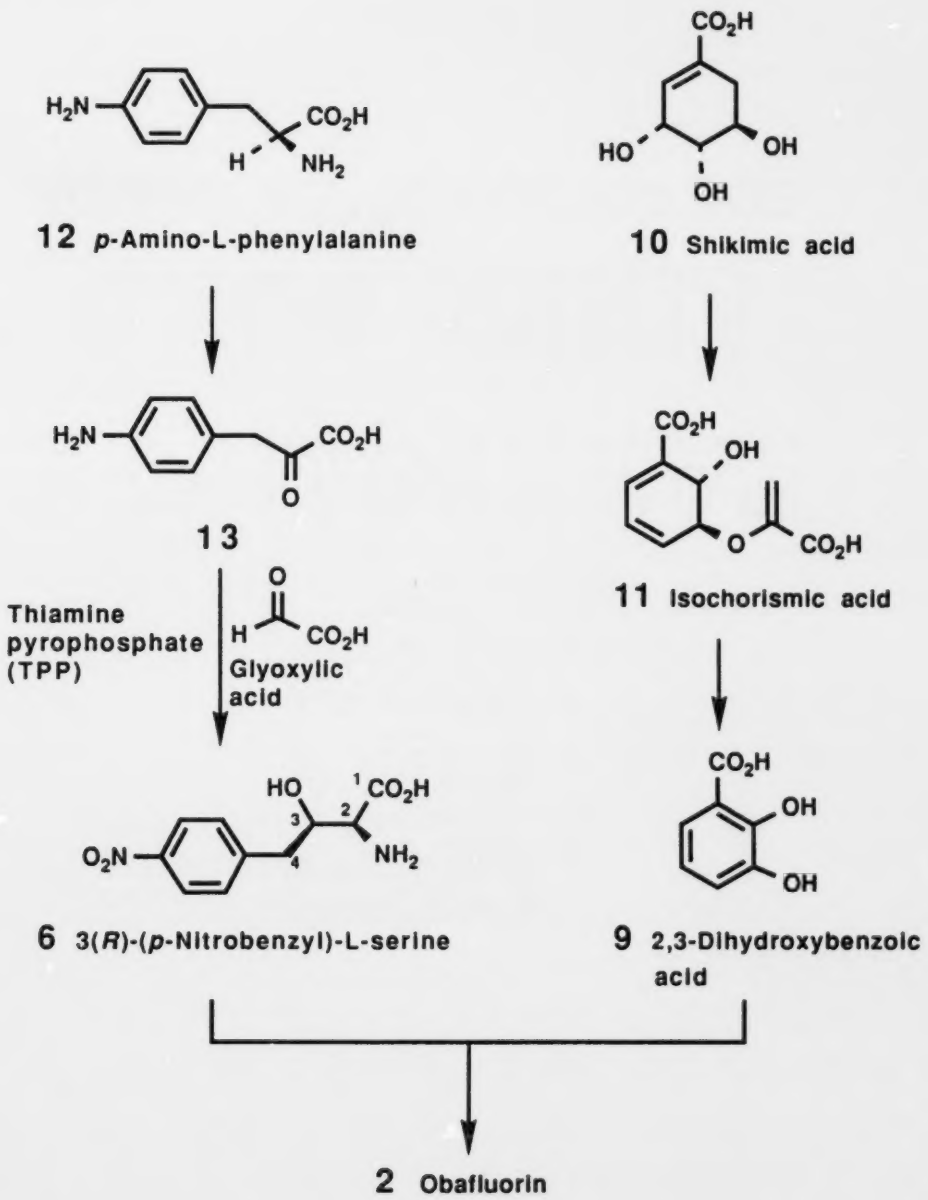
7 Chloramphenicol



8 Enterobactin

Considerable attention has focussed on the mechanism of the biosynthesis of obafluorin because of its unique structural features.[9,15,28,29] It may be that the two key precursor units, 2,3-dihydroxybenzoic acid (**9**) and 3(*R*)-(*p*-nitrobenzyl)-L-serine (**6**) are linked together (Figure 3). Incorporation experiments using D-[U-^{13}C]glucose suggest that the 2,3-dihydroxybenzoyl moiety originates from a modified shikimate pathway through the intermediate, isochorismic acid (**11**).[9,15] The mechanism of the biosynthesis of 3(*R*)-(*p*-nitrobenzyl)-L-serine (**6**) is relatively complicated. Incorporation experiments using L-phenylalanine and its *p*-nitro and *p*-amino

Figure 3. Biosynthesis of obafluorin (2).



derivatives show that *p*-amino-L-phenylalanine (**12**) is the key precursor for **6**, whereas L-phenylalanine and *p*-nitro-L-phenylalanine are poorly utilized.[9,15] This result is similar to the biosynthesis of the antibiotic chloramphenicol (**7**) where the key precursor is also *p*-amino-L-phenylalanine (**12**), and neither L-phenylalanine nor *p*-nitro-L-phenylalanine is involved.[9,15] Further incorporation studies indicate that C-1 and C-2 in 3(*R*)-(*p*-nitrobenzyl)-L-serine (**6**) originate specifically from an intact molecule of glyoxylic acid instead of glycine.[28,29]

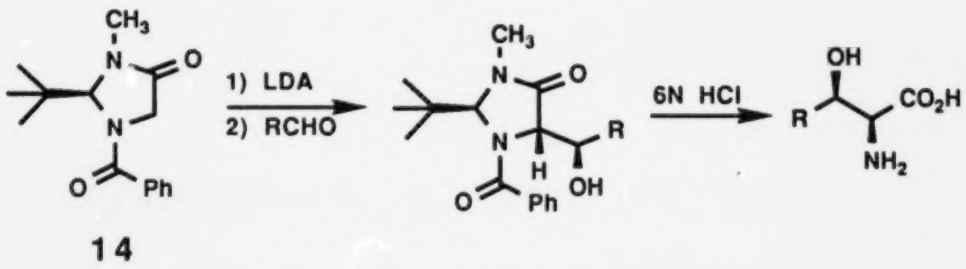
The unusual structural features and unprecedented biological activity of obafluorin (**2**) inspired us to explore its total synthesis. Since the earlier preparation of the simple β-lactone antibiotic SQ 26,517 (**1**) only gave the racemic product in low yield (Figure 2),[6] we also wished to devise an efficient stereoselective synthesis for the compounds of this class. Thus, the primary goal of the present work was to develop a general approach to the synthesis of β-lactone antibiotics, such as **1** and **2**, to study the structure-activity relationships of β-lactone antibiotics, and to develop new and more powerful β-lactone drugs by rational structural modification.

2. Approaches to the Syntheses of α-Amino β-Lactones.

Many approaches can be used to construct a β-lactone ring,[30-32] but the number of methods to synthesize α-amino β-lactone derivatives is limited, the most common approach being the cyclization of protected β-hydroxy α-amino acids.[6,33-40]

L-Serine and L-threonine are the two most common β-hydroxy α-amino acids, and over the last decade, a large number of methods have been developed for the stereoselective synthesis of unusual α-amino acids of this type.[41-60] For example, Seebach and co-workers[45-48] employed an asymmetric aldol condensation of the enolate derived from an imidazolidinone chiral auxiliary **14** with various aldehydes (Figure 4). Subsequent acidic hydrolysis of the aldol adducts generates β-hydroxy α-amino acids with excellent diastereoselectivity (d.e.>95 %).

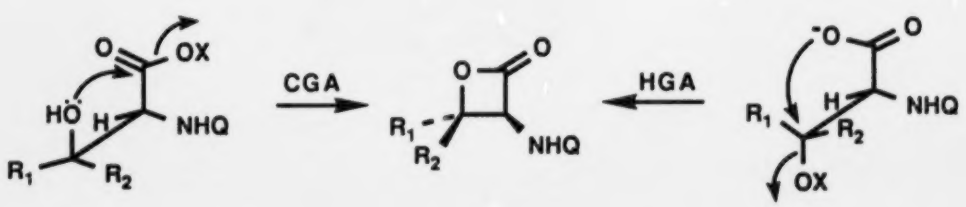
Figure 4. Synthesis of β-hydroxy α-amino acids using Seebach's approach.



14

β-Lactone formation from β-hydroxy α-amino acids can be generally achieved through two pathways, carboxyl group activation (CGA)[6,33-38] and hydroxyl group activation (HGA).[6,39,40,61-67] Cyclization through hydroxyl group activation followed by intramolecular S_N2 displacement is accompanied with a configuration inversion at the β-position, whereas carboxyl group activation followed by lactonization proceeds with the retention of stereochemistry (Figure 5).

Figure 5. α-Amino β-lactone formation from β-hydroxy α-amino acids.



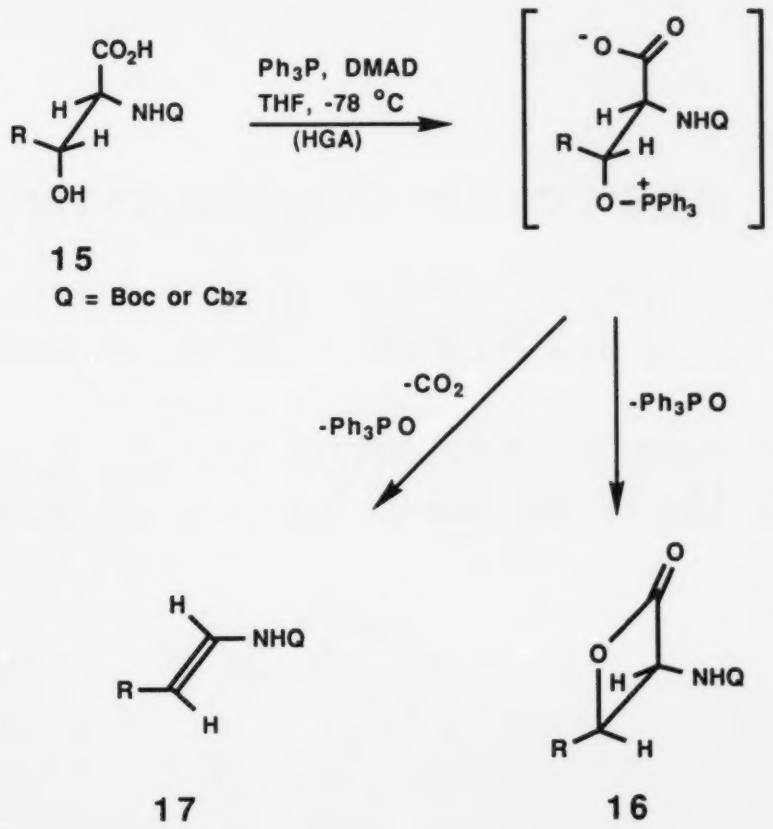
OX: Leaving Group
Q: Protecting Group

Prior to 1985, syntheses of *N*-protected α-amino β-lactones employing carboxyl group activation[6,33,34] (e.g., carbodiimide reagents) typically gave yields ranging from 26% (*N*-trityl)[33] to 1% or less (*N*-acyl).[6,33] Alternative methods involving the generation of a leaving group at the β-position (HGA)[6,39,40] also proceeded in low yields with the exception of Hofmann rearrangement and the subsequent diazotization of (benzenesulfonyl)asparagines, which afforded α-(benzenesulfonamido) β-lactones in up to 45% overall yields.[39,40] However, this latter method appears to be restricted to use of benzenesulfonyl protecting groups.[61]

The most successful recent (≥1985) approach to the synthesis of serine β-lactones involves cyclization of *N*-alkyloxycarbonyl (Q = Boc or Cbz) serine (**15**, R = H) under modified Mitsunobu conditions (Ph_3P, dimethyl azodicarboxylate (DMAD), -78 °C), a method developed by Vederas and co-workers,[61-67] to provide serine β-lactone **16** stereospecifically in consistently good yields (60-72%) (Figure 6). The mechanism of β-lactone formation under these conditions was studied in detail by isotope-labelling experiments.[62] The results show that, in contrast to alkyl substituted β-hydroxy acids,[68,69] the ring closure of serine derivatives at -78 °C proceeds by hydroxyl group activation. This results in displacement of the oxygen at C-3 and inversion of configuration at that site to give a β-lactone product. The olefin (**17**, R = H) resulting from the decarboxylative elimination is also formed as a minor product, but its amount can be kept low by careful control of reaction conditions, e.g., low temperatures. The β-lactone product obtained by this method, either *N*-Boc or *N*-Cbz serine β-lactone, has proved to be a very useful synthetic intermediate in the preparation of novel β-substituted α-amino acids through a nucleophilic ring opening at the β-position (see Section 3, Figure 12).[61,63-65,70-72]

In addition to the two examples of direct cyclization of *N*-acetyl threonine to form the corresponding threonine β-lactone **1** (SQ 26,517) in low yields[6] discussed above (Figure 2), threonine β-lactone formation has been studied under various

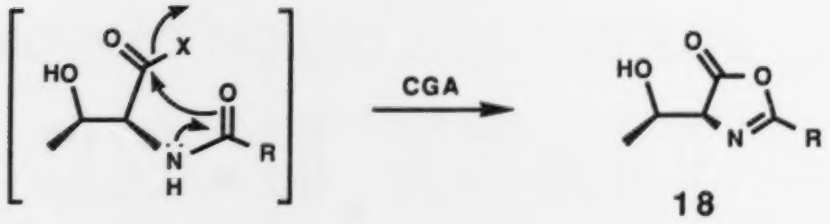
Figure 6. Synthesis of β-lactones under modified Mitsunobu conditions.



conditions.[36] The corresponding *N*-alkyloxycarbonyl threonine (**15**, R = Me, Q = Boc or Cbz, Figure 6) under low temperature Mitsunobu conditions gave exclusively the decarboxylative *anti* elimination product (**17**, R = Me, Figure 6) in stereospecific fashion. Apparently, the methyl group at the β-position in the phosphonium intermediate hinders the nucleophilic displacement by the carboxyl group and allows the elimination process to dominate. Hence formation of β-substituted α-amino β-lactones seems to require a reagent that gives carboxyl group activation. However, all attempts to cyclize *N*-protected threonine derivatives having a carbonyl group directly attached to

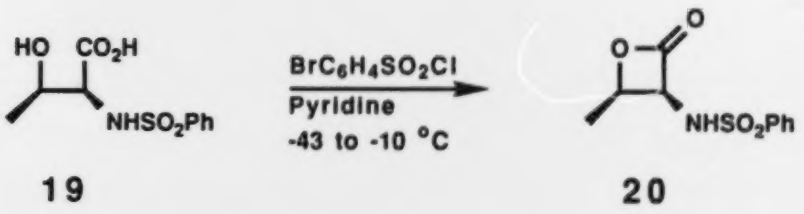
nitrogen (e.g., Boc or Cbz) gave no β-lactone product if carboxyl group activation was used, presumably due to the competing formation of azlactone **18**[36,73-75] (Figure 7).

Figure 7. Azlactone formation.



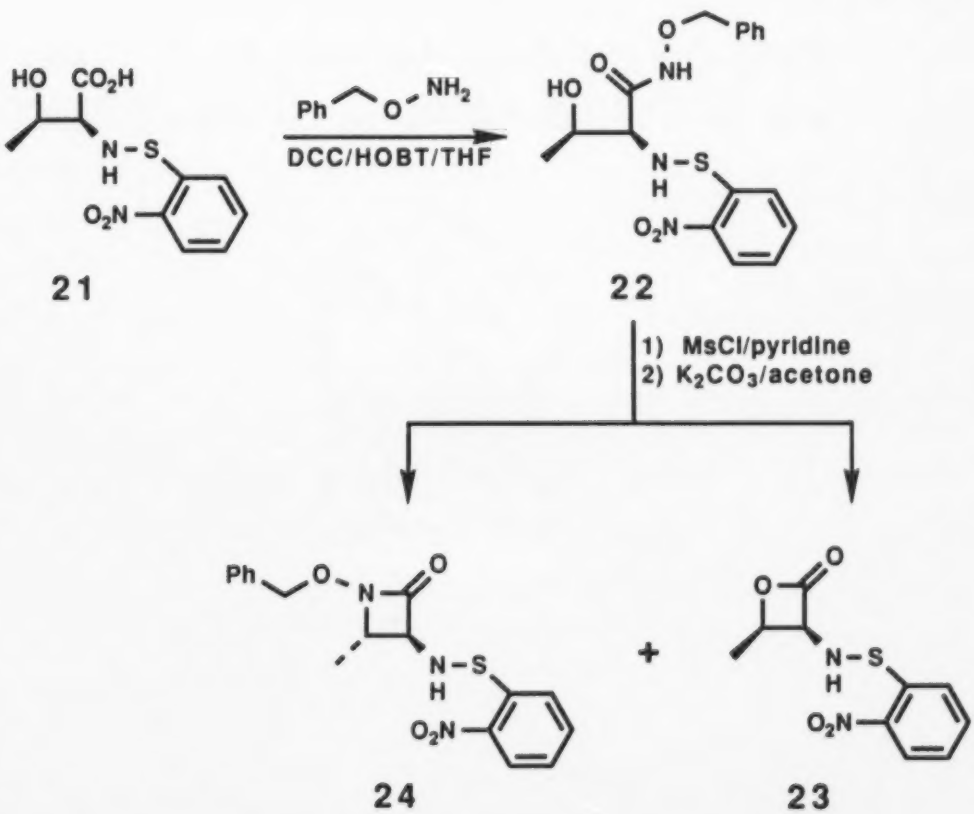
The use of a benzenesulfonyl protecting group avoids this problem and allows the formation of threonine β-lactone **20** in reasonable yields (40-55%) by carboxyl group activation with 4-bromobenzenesulfonyl chloride in pyridine (Figure 8).[36] However, the *N*-benzenesulfonyl protecting group is inconvenient because of the drastic conditions required for its removal (e.g., Na/NH_3 or refluxing HBr).[37]

Figure 8. Synthesis of *N*-benzenesulfonyl L-threonine β-lactone.



Interestingly, during a study on the syntheses of monobactam compounds (e.g., **24**), the *N*-protected L-threonine bearing an (*o*-nitrophenyl)sulfenyl group on nitrogen **21** was inadvertently cyclized in three steps to the corresponding β-lactone **23** in 8% yield (Figure 9).[35] This has prompted us to investigate the use of this protecting group for formation of β-substituted α-amino β-lactone derivatives. In the present

Figure 9. β-Lactone formation from L-threonine bearing an (*o*-nitrophenyl)-sulfenyl group.



work, we describe:

(1) the development of a general methodology for the synthesis of β-lactone antibiotics employing *N*-(*o*-nitrophenyl)sulfenyl protected β-hydroxy α-amino acids as starting materials. This method is applied to the syntheses of SQ 26,517 (**1**) and its analogs from *N*-(*o*-nitrophenyl)sulfenyl L-threonine;[37]

(2) the first total synthesis of optically pure (+)-obafluorin (**2**) and the syntheses of several α-amino-β-lactone tosylate salts bearing a *para*-substituted benzyl group at the β-position;[38]

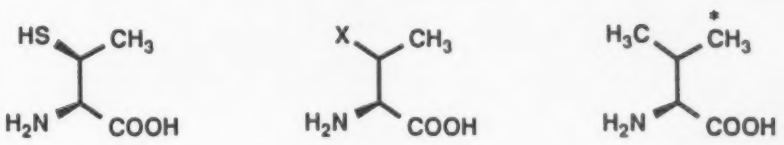
(3) the syntheses and antibiotic activities of a number of new *N*-acyl α-amino β-lactones based on modifications of the structural features of obafluorin.

3. β-Lactones as Synthetic Intermediates.

Over the last a few decades, a large number (>700) of α-amino acids has been discovered in nature, and many more have been produced synthetically.[76-78] While the 20 common L-α-amino acids are the basic building blocks of proteins and peptides that are indispensable for life, the non-proteingenic amino acids, occurring either in free form or as constituents of larger molecules, are responsible for a wide spectrum of biological activities.[76-80] Amino acids also provide an enormous pool of optically pure chiral units for organic chemists, who use them and their derivatives as chiral synthons, catalysts, and auxiliaries in asymmetric syntheses.[41,61-67,76,78,81-91]

β-Substituted and β-disubstituted α-amino acids are the constituents of important antibiotics and physiologically active peptides.[77,78,92-95] Several β-methyl α-amino acids are especially significant (Figure 10). For example, 3-methylcysteine (**25**) is a component of β-methyllanthionine, a constituent amino acid of the peptide antibiotic nisin.[96-98] β-Halo-α-aminobutyrates (**26**) have been employed in several biological investigations and mechanistic studies of enzymatic reactions.[99-107] Studies

Figure 10. Examples of β-disubstituted α-amino acids.

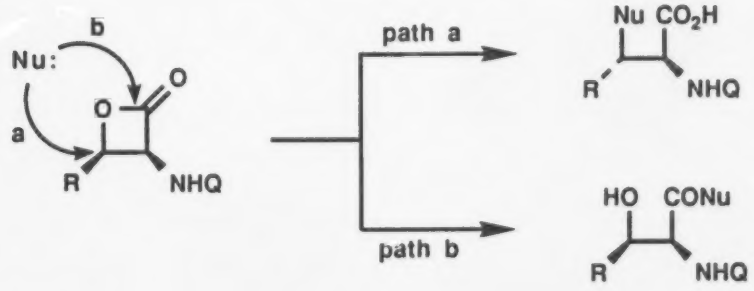


25 **26 (X = Cl, Br, I)** **27**

of penicillin biosynthesis[110-111] have specifically used labelled valines (e.g., **27**).[108-109]

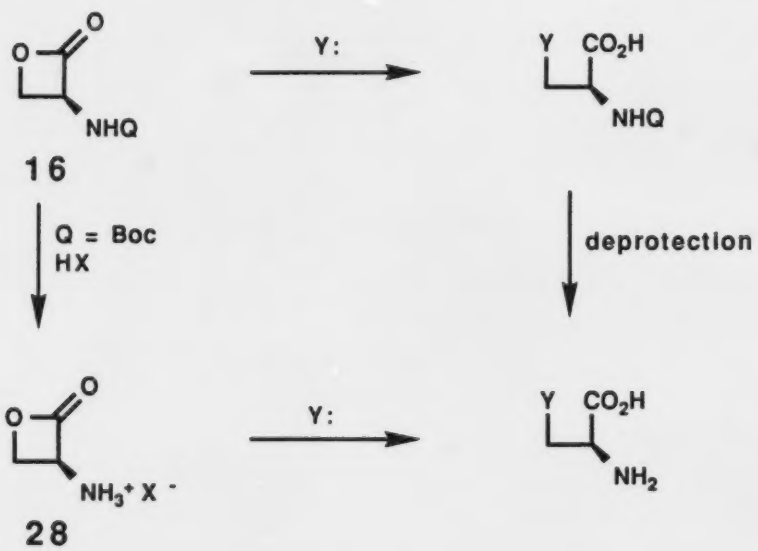
Much recent work has focussed on enantioselective syntheses of α-amino acids.[113-133] A very attractive approach to the synthesis of β-substituted or β-disubstituted α-amino acids involves the nucleophilic ring opening of α-amino β-lactones at the β-position (Figure 11, path **a**).[30-32,76-78,134] The reactivity of the β-lactone ring is unique due to the small-angle strain (23 kcal·mol^{-1}).[135-136] In addition, nucleophilic attack can proceed on the carbonyl group with acyl-oxygen

Figure 11. General pathways for nucleophilic ring opening of β-lactones.



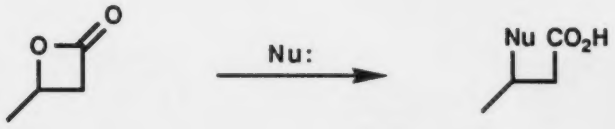
cleavage (path **b**) to give β-hydroxy α-amino acid derivatives (e.g., peptide formation[39,40]). For serine β-lactones (Figure 11, R = H), "hard" nucleophiles (e.g., hydroxide, alkoxide, alkyllithium) attack the carbonyl group whereas "softer" anions displace at the β-position.[36,61,63] Treatment of *N*-alkyloxycarbonyl (e.g., Boc or Cbz) serine β-lactones **16** or the corresponding deprotected derivatives **28** (X = CF_3CO_2 or TsO) with a variety of nucleophiles (Y:), such as halogens, heteroatom (oxygen, nitrogen, or sulfur) nucleophiles, and organolithium-derived cuprate reagents, results in a number of novel β-substituted α-amino acids in excellent yields without racemization

Figure 12. Nucleophilic opening of protected and deprotected L-serine.



(Figure 12).[61-67] Since β-lactones derived from 3-hydroxybutyric acid react with nucleophiles selectively at the β-position (Figure 13),[137,138] potentially the threonine β-lactones could undergo similar transformation to generate β-disubstituted α-amino acids (e.g., **25**, **26** and **27**, Figure 11). However, in contrast to the facile ring opening of serine β-lactones, the *N*-benzenesulfonyl L-threonine β-lactone (**20**) reacts with only a few nucleophiles, such as halides and thiourea, at the β-position.[36]

Figure 13. Nucleophilic ring opening of β-butyrolactones.

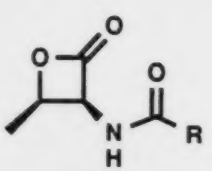


A transformation involving an S_N2' ring opening of the simple β-vinyl- and β-ethynyl-β-propiolactones with organocopper reagents has been reported by Sato *et al.*[139,140] The extension of this approach to the synthesis and ring opening reactions of β-vinyl-α-amino-β-lactones **29** would provide an access to β,γ-unsaturated amino acids (Figure 14), some of which are natural products possessing antibiotic and enzyme inhibitory properties.[141-147] However, the work by Vederas and Pansare showed that the synthesis of the β-vinyl β-hydroxy amino acid using Seebach's approach (Figure 4, R = $CH{=}CH_2$) and the subsequent *N*-protection and lactonization are difficult, presumably due to the sensitivity of these α-amino β-vinyl compounds.[95] The corresponding β-lactone bearing an *N*-benzenesulfonyl protecting group (**29**, Q = SO_2Ph) is also unstable.[95]

Figure 14. S_N2' ring opening of a β-vinyl α-amino β-lactone.

29

In the following section, we also report the results of studies on the nucleophilic ring opening of the L-threonine β-lactone derivatives, including both the *N*-protected and the *N*-deprotected L-threonine β-lactone intermediates involved in the synthesis of SQ 26,517 (**1**) as well as the *N*-benzoylated analog of **1**.[37]



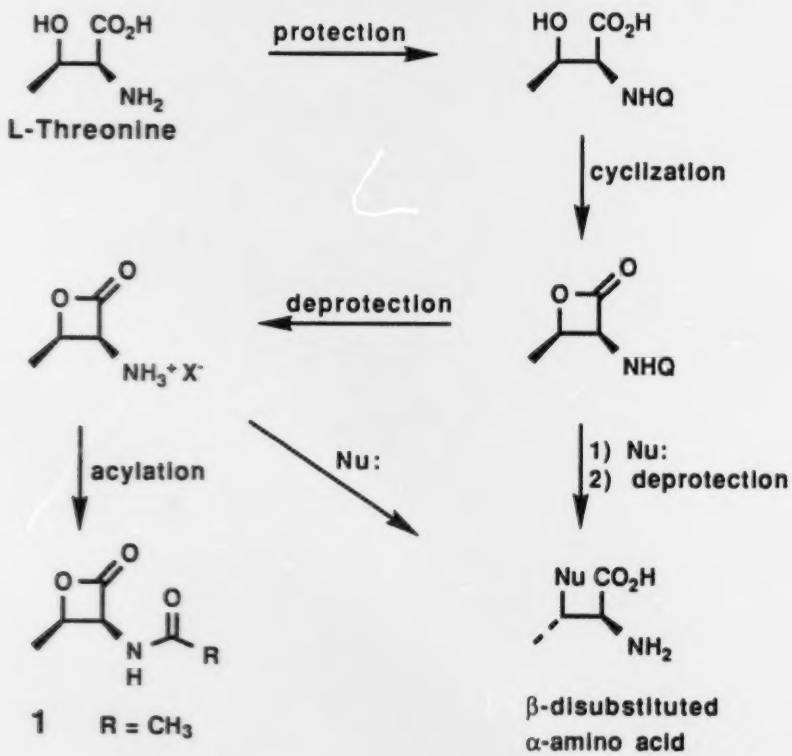
1 R = CH_3
R = Ph

Results and Discussion

Part 1. Syntheses of SQ 26,517 (1) and Other *N*-Acylated Threonine β-Lactones

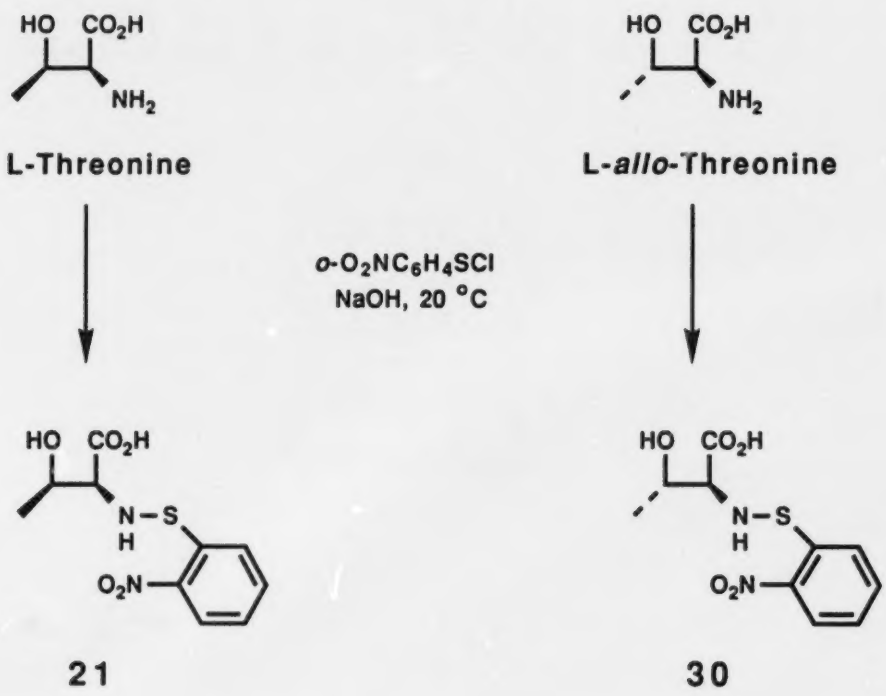
Our research on the syntheses of threonine β-lactones had two objectives: first, to develop a general approach for the syntheses of SQ 26,517 (**1**)[5,6] and other β-lactone antibiotics (mostly *N*-acyl α-amino-β-lactones);[7-12] and second, to explore the reactivity of threonine β-lactones with a variety of nucleophiles at the β-position to give β-disubstituted α-amino acids, some of which are the constituents of important antibiotics[92-95] (Scheme 1).

Scheme 1.



Previously reported syntheses of L-threonine β-lactone derivatives either proceeded in very low yields[6] or gave a product bearing an inconvenient *N*-benzenesulfonyl protecting group.[36,148,149] An isolated report in which the L-threonine derivative **21** bearing an (*o*-nitrophenyl)sulfenyl group was inadvertently cyclized to β-lactone **23** in 8% yield over 3 steps[35] (Figure 9, Chapter 1) prompted us to investigate this protecting group. Thus, treatment of L-threonine with commercially available (*o*-nitrophenyl)sulfenyl chloride and sodium hydroxide (2 N solution) in a mixed solvent (H_2O/dioxane) generates **21** in 79% yield (Scheme 2).[37] The original procedure[35] gives a crude product containing impurities that are very difficult to separate and purification by recrystallization (acetone/hexane) affords a low recovery

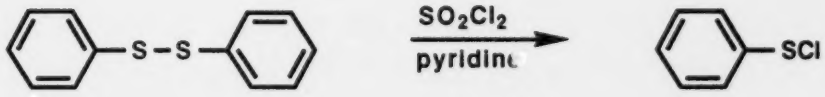
Scheme 2.



(55%).[36] Improvement of the work-up process involves first filtering the reaction mixture to remove the solid residue and then extracting the filtrate with ethyl acetate to remove any organic impurities before acidification to give **21**. The crude product from this modified procedure is almost pure (from ^{1}H NMR), and recrystallization from acetone/hexane provides analytically pure **21** as a bright yellow solid.[37] The *N*-protected L-*allo*-threonine **30** is also prepared following the improved procedure in 76% yield (Scheme 2).[37]

In order to establish whether the nitro functionality is crucial in the protecting group, phenylsulfenyl chloride **31** was prepared from phenyl disulfide (Scheme 3).[150] However, treatment of L-threonine with **31** under the basic conditions results in the vigorous hydrolysis of **31** (HCl evolution). A literature search revealed that phenylsulfenyl chloride is hydrolyzed more than 1000 times faster than its nitro

Scheme 3.



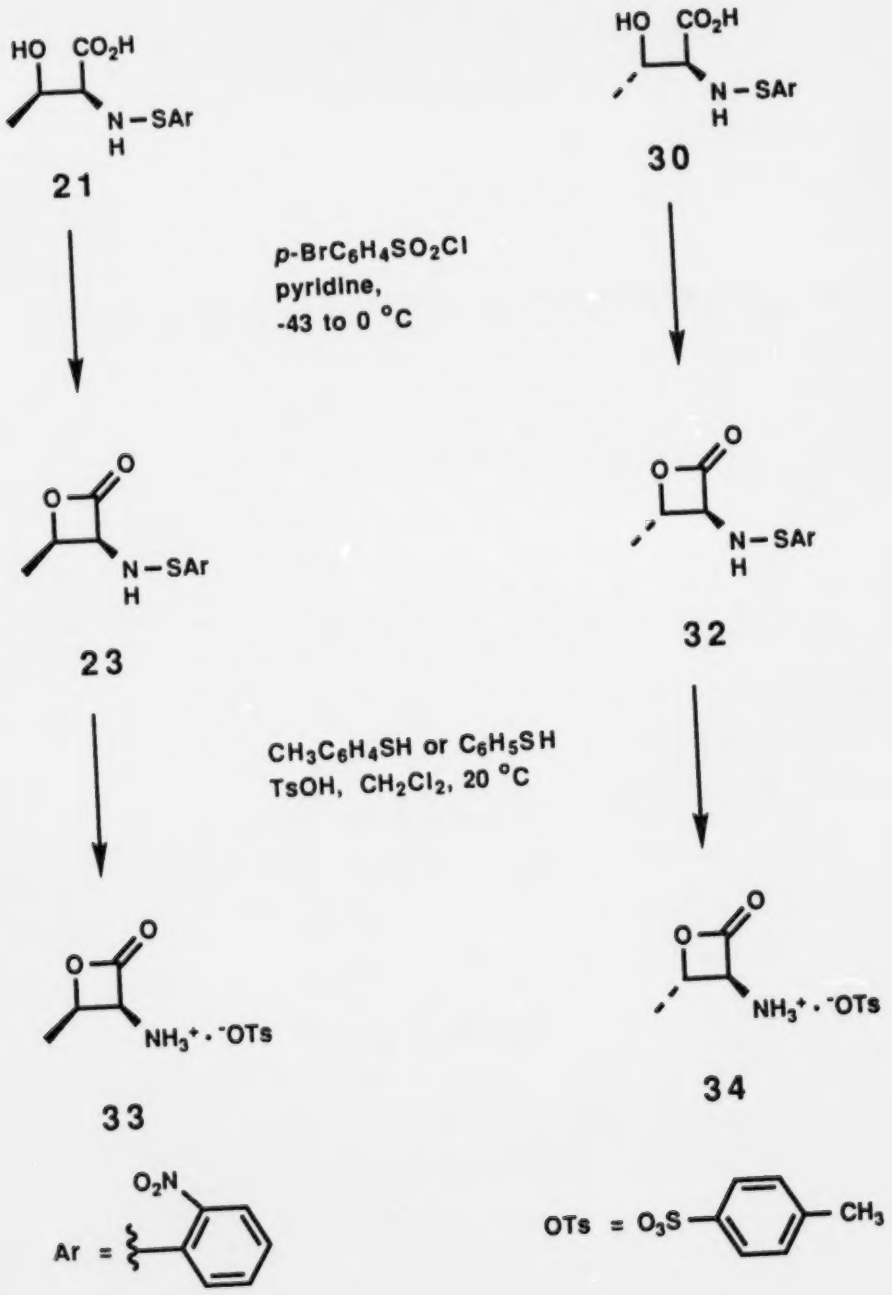
31

derivative in a chloroform solution containing water (2-3 x 10^{-2} M) and Et_4NCl (1-2 x 10^{-2} M).[151] Hence the relatively 'inert' (*o*-nitrophenyl)sulfenyl chloride seems to be an appropriate reagent under the present conditions. Furthermore, the presence of the nitro group is probably essential since its strong electron-withdrawing capability will make the protected amino group less nucleophilic, which may be important for the β-lactone ring closure.

Initial investigations on the cyclization of optically pure **21** and **30** to the corresponding β-lactones **23** and **32** (Scheme 4) indicate that the best conditions (45-56% yield) are similar to those employed earlier for the *N*-benzenesulfonyl analogs,[36] namely carboxyl group activation by 4-bromobenzenesulfonyl chloride in pyridine at low temperatures. Modification of reaction conditions (e.g., base, solvent and reagent compositions) drastically reduces the yield of the β-lactone. The purities of the starting materials, especially **21** and **30**, appear to be critical in order to achieve the highest yield. Most reactions were done at -43 to 0 °C, but the temperature range for the present transformation is relatively flexible; several trial experiments, ranging from 0 °C to room temperature afforded the β-lactones in yields of 53-55%. However, the reaction mixture should not be kept at room temperature for more than 3 h because of the slow decomposition and/or polymerization of β-lactones in pyridine. A reaction using benzenesulfonyl chloride to replace its 4-bromo derivative as the activating reagent gives the β-lactone **23** in comparable yield (45%). In the cases of small scale reactions (< 2 mmol), the work-up procedure can be simplified by removing pyridine (< 14 mL) under high vacuum and then purifying the residue directly by flash chromatography; this avoids the low temperature (0 °C) aqueous acidification process during which β-lactones may decompose.

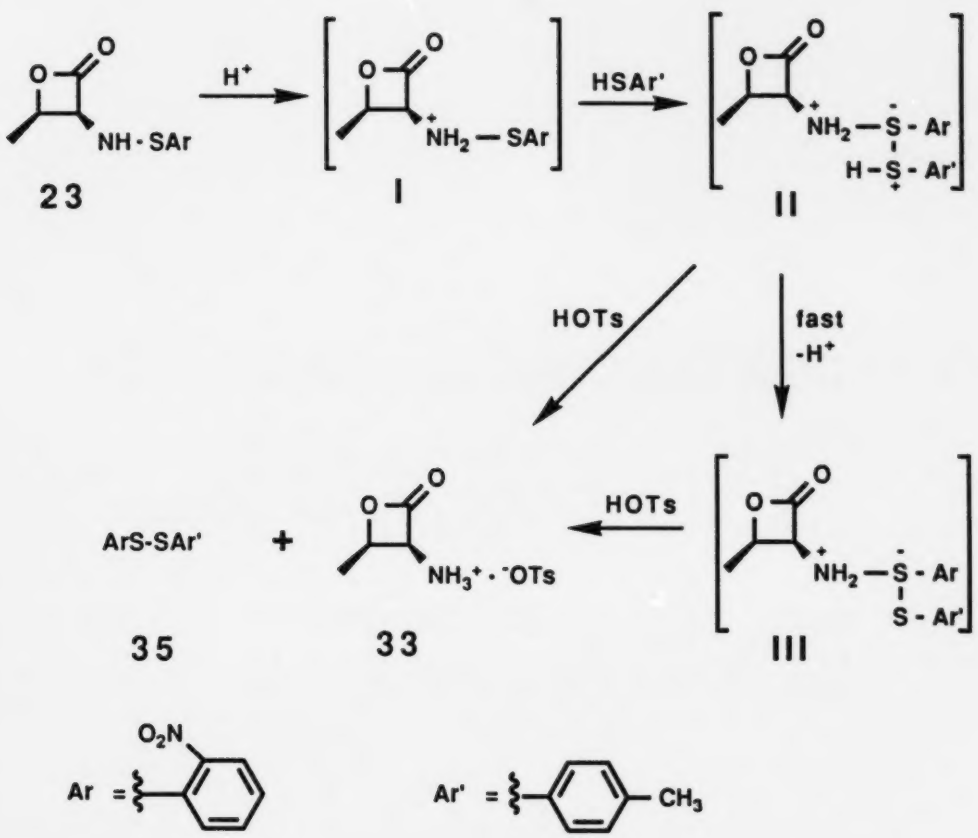
The stereochemistries of the β-lactones **23** and **32** are confirmed by their ^{1}H NMR spectra. The coupling constants between the α and β hydrogens are 6.0 Hz for the *cis* β-lactone **23** and 4.0 Hz for the *trans* β-lactone **32**. These values are in good agreement with literature values for analogous structures,[8,36,95,152] and no epimerization could be detected by ^{1}H NMR analysis (≥ 99% one isomer).

Scheme 4.


HO CO2H
N – SAr
H
21
30
p-BrC6H4SO2Cl
pyridine,
-43 to 0 °C
O
23
32
CH3C6H4SH or C6H5SH
TsOH, CH2Cl2, 20 °C
NH3+ · -OTs
33
34
O2N
Ar =
OTs = O3S
CH3

The (*o*-nitrophenyl)sulfenyl protecting group can be removed from **23** and **32** by facile thiolysis[35] with aromatic thiols such as thiophenol or *p*-thiocresol in the presence of *p*-toluenesulfonic acid (anhydrous or monohydrate) under carefully controlled conditions to afford the tosylate salts of the previously unknown parent oxetanones **33** and **34** (76-92% yield), respectively (Scheme 4).[37] High purities of **23** and **32** are crucial, since even a trace of sulfur containing impurity that reacts with the thiol reagent can lead to the failure of the reaction. The mixed disulfide **35** can be isolated from the reaction mixture by ether trituration, thus supporting the previously proposed deprotection mechanism[153] involving nucleophilic attack by the aromatic thiol group on the sulfenyl sulfur atom. Recently, a detailed study on the mechanism and kinetics of thiolysis of (*o*-nitrophenyl)sulfenamides was reported.[154] According to the results, the acid-catalyzed thiolysis shows a first-order dependence of the rate on both thiol and acid concentrations, and a two-step mechanism involving the sulfuranide intermediate (II) was proposed rather than the one where the bond-forming and bond-breaking processes are concerted. This hypervalent sulfur intermediate (II) has not yet been isolated experimentally, but the kinetic studies in several cases suggest its presence.[154] Based on these results, a mechanism for the present transformation is outlined in Scheme 5. Thus, (*o*-nitrophenyl)sulfenyl L-threonine β-lactone **23** is first protonated under acidic conditions to form (I) which is attacked by the thiol to give a hypervalent sulfuranide (II). The products are obtained from (II) through two possible routes: one by immediate cleavage of the sulfur-nitrogen bond followed by proton transfer, and the other one in the opposite order.

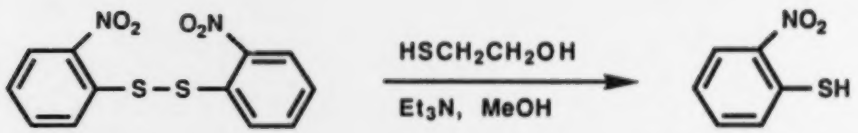
Scheme 5.



However, the actual sequence of events during the thiolysis may be considerably more complicated because reagent concentration appears to be a critical factor for the success of this reaction; modest dilution of the mixture hinders the transformation and leads to recovery of starting material. Additionally, the symmetrical disulfide **36** is also produced and may play an important role in the completion of the deprotection.[153] One of the possible complications of the deprotection reaction is that the nucleophilic thiol may affect the sensitive β-lactone group, and therefore, *o*-nitrothiophenol (**37**), a less nucleophilic aromatic thiol due to the electron-withdrawing

capability of the nitro group, was prepared (Scheme 6) from *o*-nitrophenyl disulfide (50% yield)[153] and tried as the deprotecting reagent. The deprotection of **23** with

Scheme 6.



36 **37**

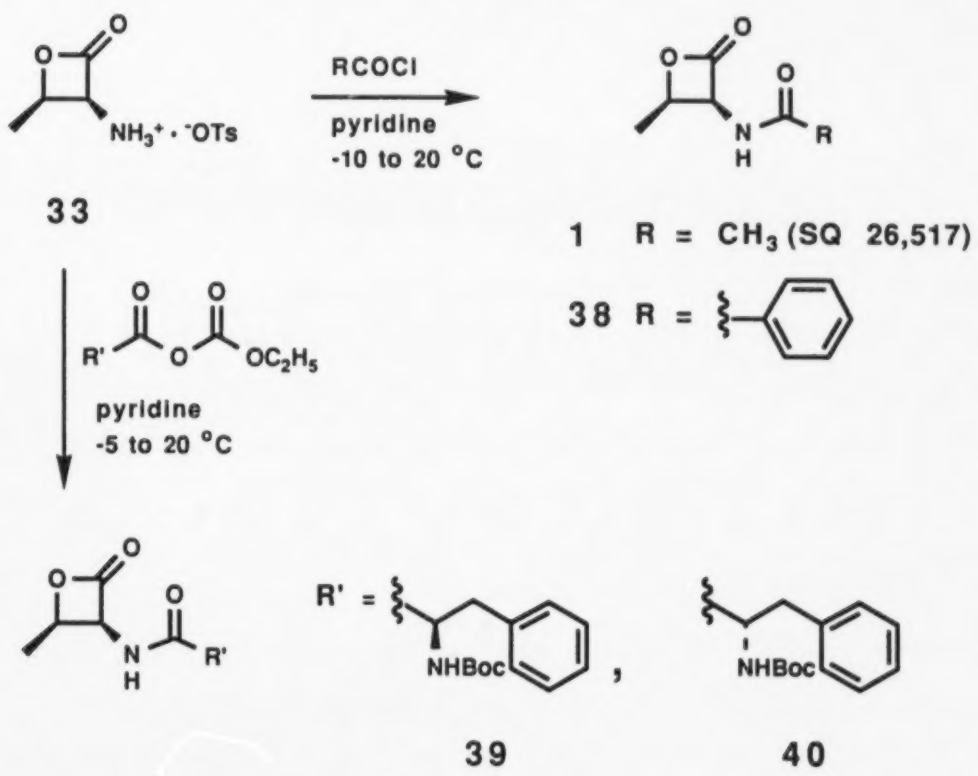
thiol **37** proceeds similarly to the reactions using *p*-thiocresol or thiophenol, but purification of the product by trituration with diethyl ether proves to be difficult because the disulfide **36** produced is only very sparingly soluble in ether. Since *p*-thiocresol is a solid and easier to handle than thiophenol, it is the reagent of choice for the deprotection.

The β-substituted salts **33** and **34** are much more stable under acidic or neutral aqueous conditions than the corresponding unsubstituted β-lactone salt derived from serine,[64] but dilute aqueous base destroys these compounds instantly.

Since the naturally occurring antibiotics are *N*-acylated β-substituted α-amino β-lactones,[5-12] the attachment of various acyl groups to the nitrogen of **33** was investigated (Scheme 7). Treatment of **33** with acetyl chloride and pyridine at -10 °C produces the antibiotic SQ 26,517 (**1**)[5,6] in 84% yield. The spectral data of the synthetic **1** are consistent with the literature values for the isolated natural product,[6] and no epimerization could be detected by ^{1}H NMR. The unoptimized overall yield of 28% over four steps from L-threonine compares favorably to the previous syntheses

described above.[6] Benzoylation of **33** with benzoyl chloride occurs analogously to give the corresponding *N*-benzoyl derivative **38** in 91% yield.

Scheme 7.

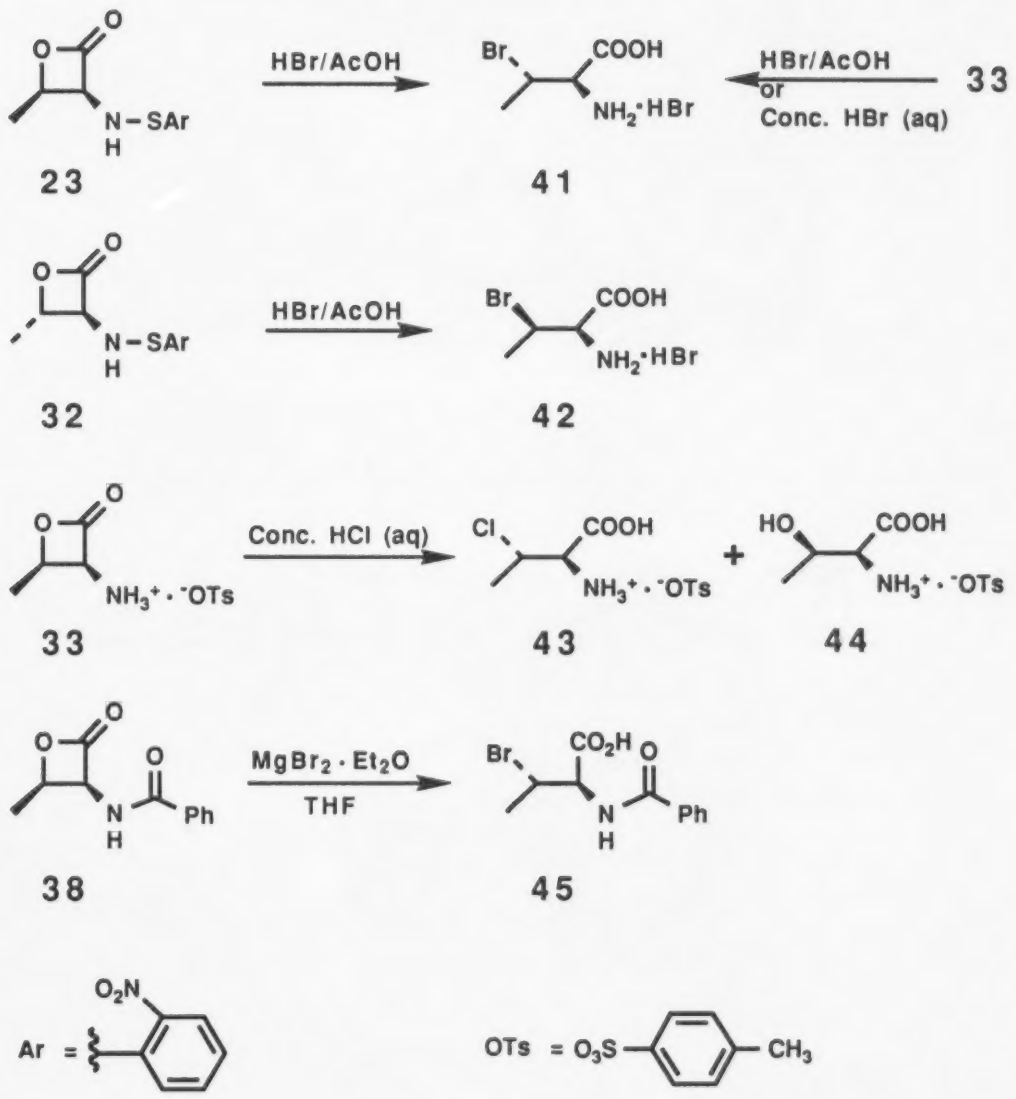


Interestingly, *N*-protected α-amino acids, and thus presumably peptides, can be attached to the β-lactone salt **33** without epimerization. Coupling of the *N-tert*-butoxycarbonyl (Boc) derivative of D-phenylalanine via the mixed anhydride[155] with ethyl chloroformate to **33** affords a 92% yield of **39**. *N*-Boc-L-Phenylalanine reacts similarly with **33** to give the other pure diastereomer **40** (92%). In such reactions it appears to be advantageous to avoid prolonged exposure of the lactone salt to base unless acylating agent is present in order to avoid complications due to lactone

decomposition and/or polymerization. Acylations of L-*allo*-threonine β-lactone tosylate **34** were also attempted with the above acylating reagents (acetyl chloride, benzoyl chloride, and mixed anhydrides of both isomers of *N*-Boc-phenylalanine); however, none of the acylated β-lactone could be obtained. The reason for this is still undetermined, but it may be that the free L-threonine β-lactone intermediate is more stable than the corresponding L-*allo* derivative.

Preliminary experiments suggest that nucleophilic attack at the β-position of the threonine and *allo*-threonine β-lactones while possible with some reagents (Scheme 8), is often disfavored, in contrast to the facile ring openings at the methylene of the serine-derived β-lactones.[61-67] This is in accord with previous observations with *N*-benzene-sulfonyl threonine β-lactone.[36] For example, attempts to open the *N*-benzoyl threonine β-lactone **38** with pyrazole, acetate, or copper-catalyzed Grignard reagents (e.g., EtMgCl with $CuBr{\cdot}SMe_2$) fail to produce significant amounts of β-substituted products. Similarly, the reaction between pyrazole and β-lactone tosylate salt **33** gives no β-substituted amino acid. However, treatment of *N*-(*o*-nitrophenyl)sulfenyl-protected β-lactones **23** or **32** with concentrated HBr in acetic acid cleaves the ring with inversion of configuration at C-3 and concomitant removal of the protecting group to give the optically pure hydrobromide salts of the 2-amino-3-bromobutanoic acids **41**[156] (68%) and **42** (69%), respectively. The stereochemical assignment relies upon comparison of chemical shifts and coupling constants for the C-2 and C-3 hydrogens for a series of *allo*-threonine and threonine derivatives.[36,95] Similar conditions (HBr in acetic acid) also transform the lactone salt **33** to **41** in 92% yield. Reaction of **33** with concentrated aqueous HBr solution also affords **41** (quantitative) without detectable epimerization, but similar treatment of **33** with concentrated aqueous HCl solution gives a mixture of the *p*-tosylate salts of 2-amino-3-chlorobutanoic acid **43** (78%) and L-threonine **44** (22%). The *N*-benzoyl β-lactone **38** is converted in high yield (94%) to the corresponding β-bromo compound **45** by anhydrous magnesium bromide, but this

Scheme 8.

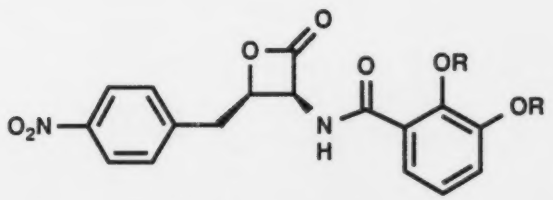

HBr/AcOH
HBr/AcOH
or
Conc. HBr (aq)
33
23
41
HBr/AcOH
32
42
Conc. HCl (aq)
33
43
44
MgBr2 · Et2O
THF
38
45
Ar =
OTs =

product is unstable at room temperature and appears to lose bromine through elimination, as shown by the appearance of signals at 5.45 (q) (3-H) and 1.42 (d) (CH_3) in the ^{1}H NMR spectrum.

Thus, a process has been developed in which *N*-(*o*-nitrophenyl)sulfenyl-protected threonine is cyclized in one step through carboxyl group activation by 4-bromobenzenesulfonyl chloride in pyridine to the corresponding protected β-lactone (45-56%) which can subsequently be deprotected (≥85%) and acylated (80-92%).[37] This approach not only allows the synthesis of the antibiotic SQ 26,517 (**1**) (28% overall yield in 4 steps from L-threonine) and some other interesting L-threonine β-lactones bearing different acyl groups (**38**, **39**, and **40**), but also clearly promises to provide ready access to a large number of other natural β-lactone antibiotics and their analogs.[37,61-67] The usefulness of the currently available threonine β-lactones for the synthesis of β-substituted α-amino acids (via nucleophilic attack on the β-position) appears to be much more limited than for the corresponding unsubstituted serine derivatives.[61-67] However, β-halo (Br or Cl) α-amino acids can be generated stereospecifically in good to excellent yields (68-100%) from the *N*-protected threonine β-lactones **23** and **32** or the *N*-deprotected tosylate salt **33** by treatment with the concentrated hydrogen halide solutions (either aqueous or in acetic acid). A number of such compounds are potent enzyme inhibitors.[99-107]

Part 2. Syntheses of (+)-Obafluorin (2) and Related β-Alkyl-α-Amino β-Lactones

Armed with the procedure developed in Part 1 for the syntheses of *N*-acyl β-lactones, it appeared feasible to synthesize the biologically more potent and structurally more challenging β-lactone antibiotic, (+)-obafluorin (**2**).[7-9] Prior to our work, a total synthesis of **2** had not been completed. Very recently Rao *et al.* reported the synthesis of racemic diacetylobafluorin (**2a**),[157] employing the methodology (cyclization, protection-deprotection) developed by our research group as described in Part 1 which had been published earlier in the *Journal of Organic Chemistry*.[37] It remains unclear whether the acetyl groups can be successfully removed from **2a** to give **2** because of the instability of pure obafluorin[8] and the general sensitivity of such α-amino-β-lactone derivatives to base and strong nucleophiles.[37]

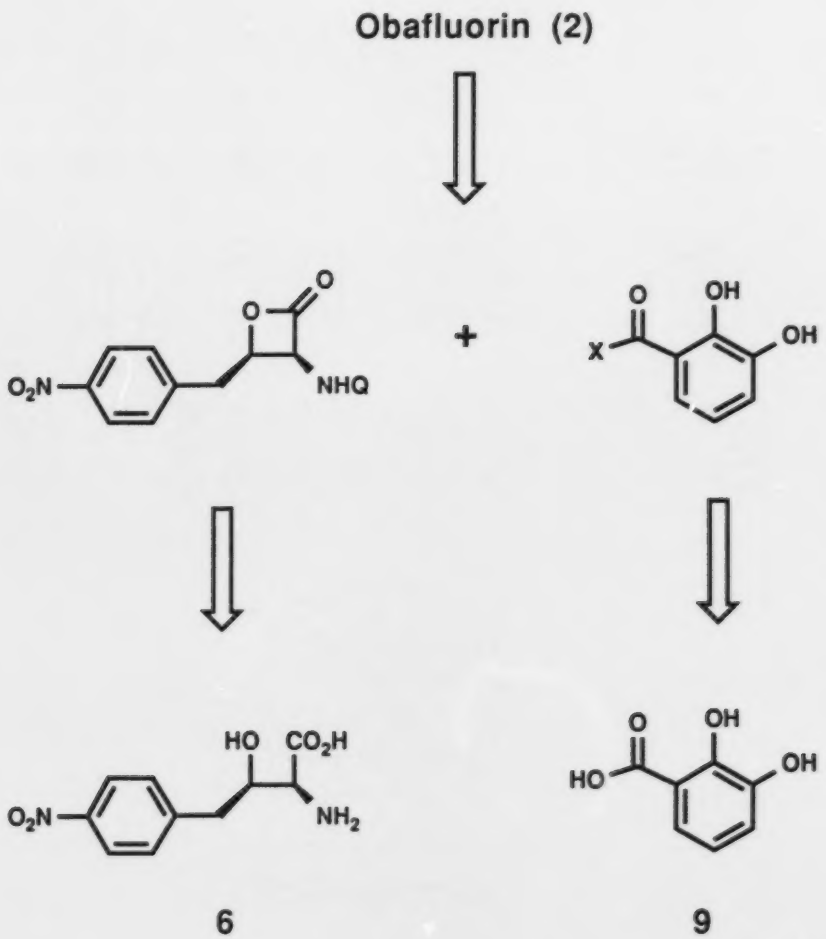


2 R = H
2a R = Ac (Racemic)

Our synthetic strategy is based on two key substructures, a (*p*-nitrophenyl)-L-threonine β-lactone derivative and a 2,3-dihydroxybenzoyl moiety (Scheme 9). The lactone unit can be further disconnected to the corresponding β-hydroxy α-amino acid **6**, which became our first target molecule. The required benzoylating agent should be readily prepared from commercially available 2,3-dihydroxybenzoic acid (**9**). Notably,

the acid **9** and the amino acid **6** are also the two key intermediates for the biosynthesis of **2** (Figure 3, Chapter 1).[9,15,28,29]

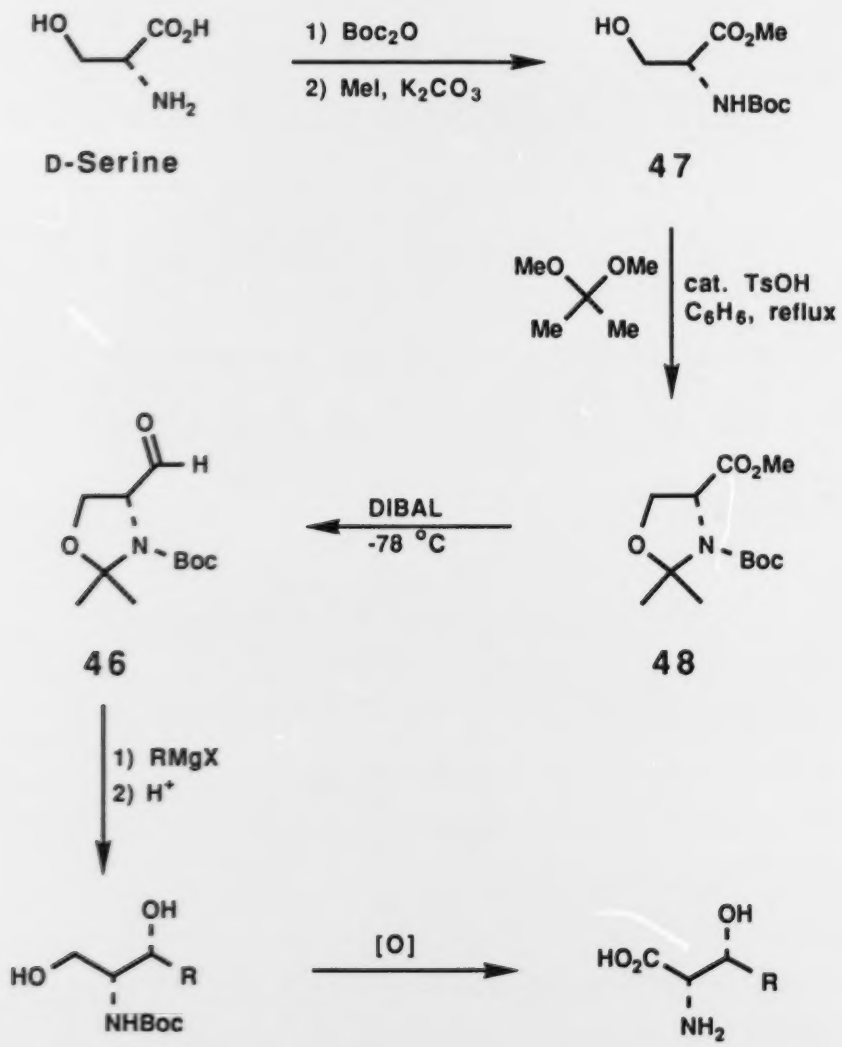
Scheme 9.



During the last decade a large number of methods have been established for the enantioselective synthesis of β-hydroxy α-amino acids.[41-60] One route developed by Garner *et al.* employs aldol condensation of the oxazolidinealdehyde **46** (a D-serinal

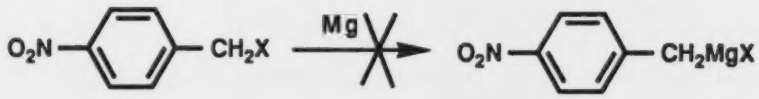
derivative) with either a Grignard reagent or an alkyllithium compound followed by acidic hydrolysis of the adduct to give an aminoethanol product with good diastereoselectivity (71-85% d.e.) (Scheme 10a).[41,58-60,158] Selective oxidation of the primary alcohol provides the *threo* β-hydroxy α-amino acid. Compound **46** can be

Scheme 10a.



prepared in three steps from inexpensive and readily available chemicals,[41,60,158] as outlined in Scheme 10a. Treatment of D-serine with di-*tert*-butyl dicarbonate (Boc_2O) at pH ≥ 10 followed by esterification with methyl iodide gives *N*-Boc serine methyl ester **47** (52% yield). The slow distillation of a solution of **47**, 2,2-dimethoxypropane (DMP), and a catalytic amount of *p*-toluenesulfonic acid (TsOH) results in the clean formation of oxazolidine **48** in 71% yield. Reduction of **48** with diisobutylaluminum hydride (DIBAL) generates the aldehyde **46** as a colorless oil (41%). As reported in the literature, the ^1H NMR spectra of both **46** and **48** show two sets of signals at ambient temperature; this is because the oxazolidine derivatives exist as slowly interconverting rotamers on the NMR time scale.[60,158] Upon raising the probe temperature to 75 °C these signals merge into one set, thus suggesting the existence of a dynamic equilibrium.[158] However, as the required Grignard reagent *p*-nitrobenzylmagnesium bromide or chloride could not be easily prepared from the corresponding halides and magnesium under a variety of conditions (Scheme 10b), this approach (Scheme 10a) was not investigated further .

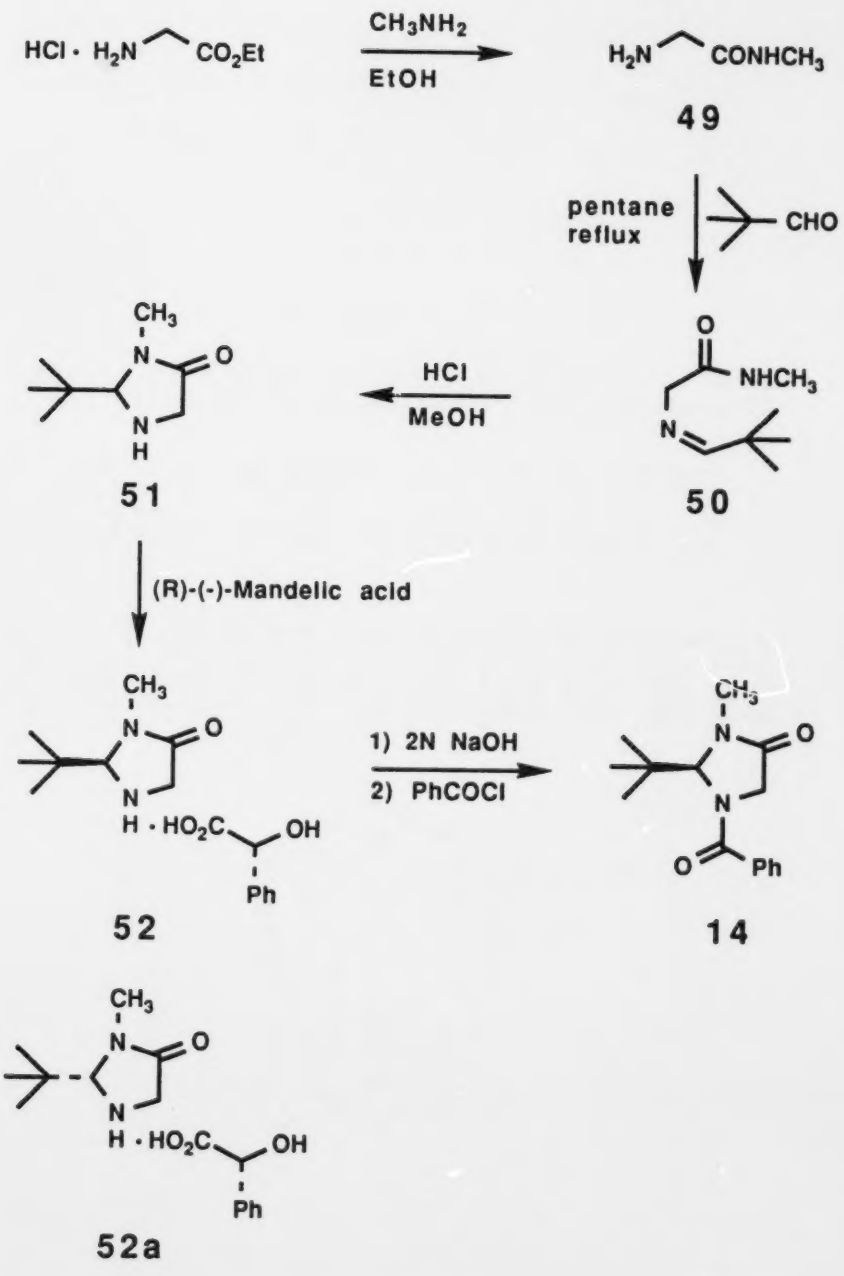
Scheme 10b.



X = Cl or Br

The imidazolidinone **14** devised by Seebach and co-workers[45-48,159,160] is an excellent chiral glycine synthon, which can be used to generate β-hydroxy α-amino acids with very high diastereoselectivity (d.e. > 95%) by aldol condensation of its enolate with various aldehydes followed by acidic hydrolysis (Figure 4, Chapter 1).

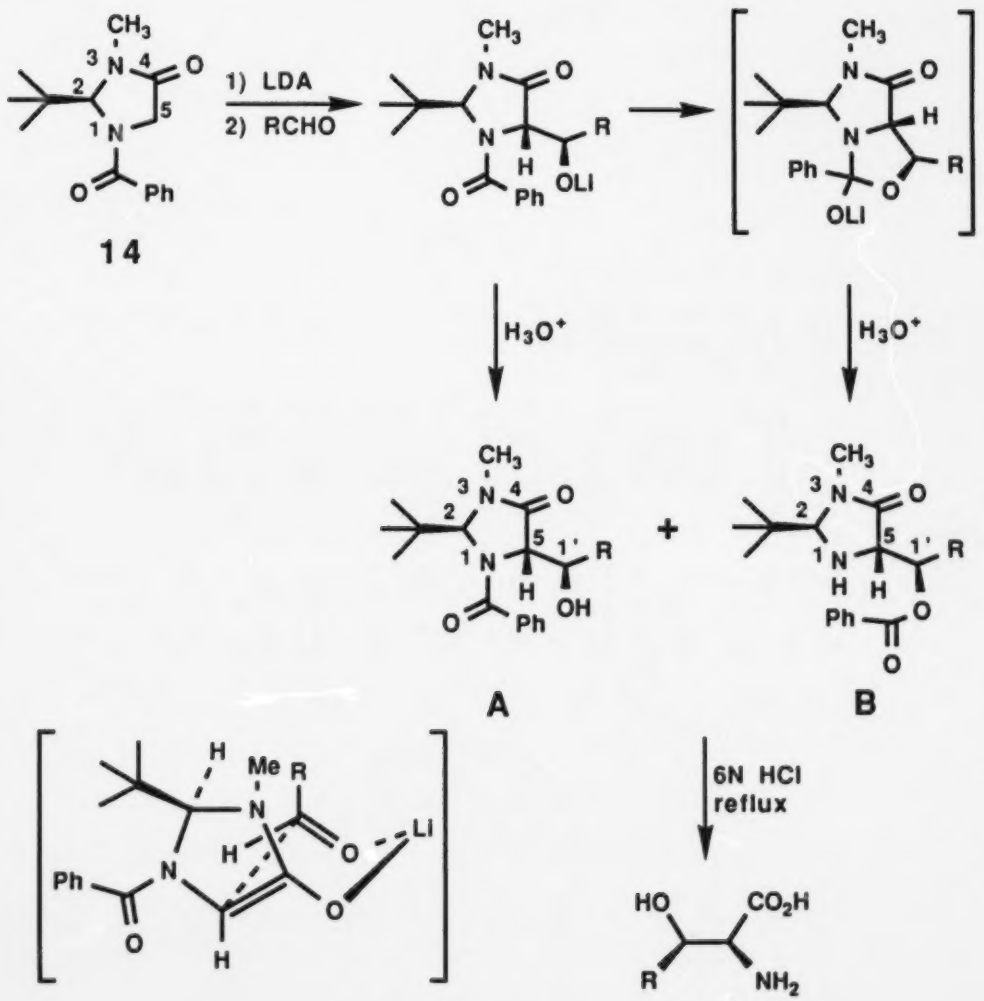
Scheme 11.


HCl · H2N CO2Et
CH3NH2
EtOH
H2N CONHCH3
49
pentane
reflux
CHO
O
NHCH3
N
50
HCl
MeOH
CH3
N
O
N
H
51
(R)-(-)-Mandelic acid
CH3
N
O
N
H · HO2C OH
Ph
52
1) 2N NaOH
2) PhCOCl
CH3
N
O
N
O
Ph
14
CH3
N
O
N
H · HO2C OH
Ph
52a

Compound **14** can be synthesized in 5 steps according to the literature procedure (Scheme 11).[159,160] Treatment of commercially available glycine ethyl ester hydrochloride with methylamine gives *N*-methylglycinamide (**49**) in 98% yield. Condensation of the amide with pivaldehyde affords the imine **50** (80%) which cyclizes upon exposure to a saturated solution of HCl in methanol to the racemic imidazolidinone **51** (66%). The racemate is then resolved by addition of (*R*)-(-)-mandelic acid. Two diastereoisomeric salts **52** (*R*,*R*) and **52a** (*R*,*S*) are formed in a boiling saturated acetone solution, but only **52** crystallizes when the solution is slowly cooled to room temperature over 6 h. The two diastereoisomers are separated by filtration. Treatment of **52** with sodium hydroxide solution affords a chiral cyclic amine intermediate (the (*R*) isomer of **51**) which is benzoylated to give optically pure **14** in 86% yield.[159,160]

The imidazolidinone **14** is usually treated with LDA to generate the corresponding enolate, which can then undergo stereospecific aldol condensation with aldehydes at low temperature (-78 to -100 °C). The two new chiral centers (C-5 and C-1') in the aldol adduct (**A**) (Scheme 12) are generated with very high stereoselectivity (d.e. > 95%). The stereocontrol at C-5, *trans* to the *tert*-butyl group, can be rationalized by the preferential approach of the aldehyde carbonyl to the enolate from the less hindered face *anti* to the *tert*-butyl group.[139] The high stereoselectivity of C-1' can be explained by a chair-type six-membered transition state proposed by Seebach and co-workers (Scheme 12).[45-48] The preference of the R group on the aldehyde to take an axial rather than an equatorial position in the chair presumably results from an unfavorable interaction between the *N*-benzoyl group and the substituent at the corresponding equatorial position.[45-48] The aldol condensation generally gives a mixture of the normal aldol adduct **A** and its rearranged isomer **B** (Scheme 12).[45,161] The rearrangement from hydroxyamide **A** to aminoester **B** occurs through a tetrahedral intermediate. The two isomers can be distinguished from their ^{1}H NMR spectra which

Scheme 12.



show different chemical shifts and coupling patterns for the protons on the imidazolidinone ring and the 'aldol' carbon (C1') (Table 1).[45] The composition of **A** and **B** in the mixture of the aldol adducts depends on the structure of the aldehyde and on both the reaction and work-up conditions. Seebach *et al.*[45] obtained mostly the rearranged adduct **B** in their reactions by quenching the reactions at room temperature.

The separation of **A** and **B** appears to be difficult by column chromatography despite their obviously different structures.[161] However, since both **A** and **B** are hydrolyzed in refluxing 6N HCl solution to the same β-hydroxy α-amino acid, they can be used as a mixture without separation.[45,161]

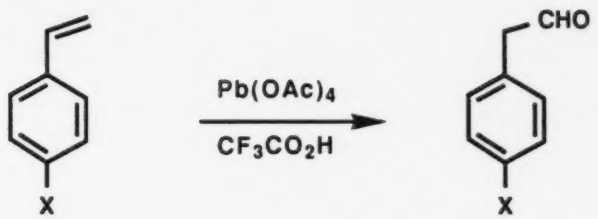
Table 1. ^{1}H NMR chemical shifts and coupling pattern of the protons at the stereogenic centers C (2), C (5), and C (1')

Adducts	δ (ppm) (coupling pattern)		
	H-2	H-5	H-1'
A	5.50-6.00 (s)	4.40-4.60 (d)	3.30-3.50 (m†)
B	4.20-4.25 (m)	3.80-4.15 (m)	5.40-6.40 (m†)

† Exact pattern depends on the number of H's on C(2').

The preparation of (*p*-nitrophenyl)acetaldehyde (**53**), required for the aldol condensation in the above approach, was attempted using several oxidation methods. The oxidation of 2-(*p*-nitrophenyl)ethanol under Swern conditions ($(COCl)_2$, DMSO, and Et_3N)[162] or using *n*-Pr_4NRuO_4 and 4-methylmorpholine *N*-oxide[163] failed to produce the aldehyde product. However, treatment of *p*-nitrostyrene with lead tetraacetate in trifluoroacetic acid (TFA) provides (*p*-nitrophenyl)acetaldehyde (**53**) in excellent yield (≥85%) and purity.[164] The mechanism of this reaction is not fully understood, and a carbocation intermediate may be involved.[164] (*p*-Chlorophenyl)-acetaldehyde (**54**) could also be prepared using the same method in 95% yield.[164]

Scheme 13.

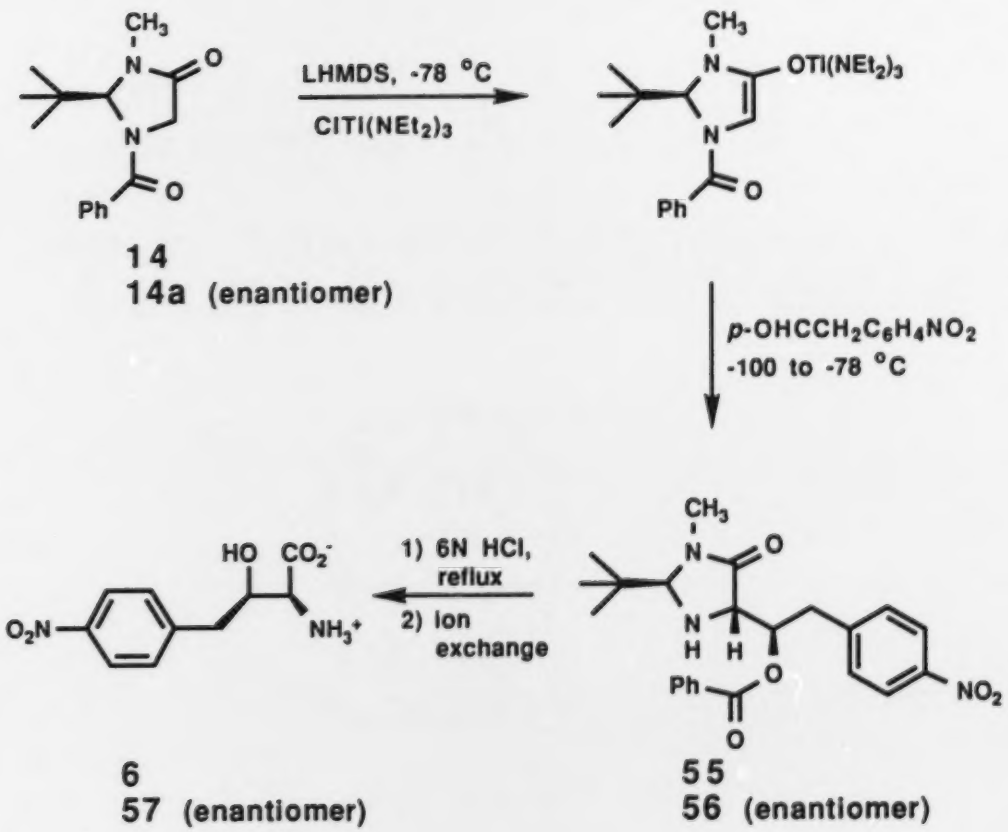


53, X = NO_2
54, X = Cl

In initial trials of the aldol condensation, lithium diisopropylamide (LDA) was used as the base for formation of the enolate from **14**, but subsequent condensation with **53** generally gave low yields (17-30%) of the aldol adducts. This is probably due to the interaction between the excess LDA (1.1-1.2 equivalent used) or the diisopropylamine generated *in situ* and the sensitive acidic aldehyde **53**. Hence, the more bulky and less nucleophilic lithium hexamethyldisilazide (LHMDS)[165] was selected. Treatment of **14** with LHMDS at -78 °C generates the corresponding enolate, which reacts with (*p*-nitrophenyl)acetaldehyde (**53**) to give the rearranged adduct **55** as the only major product. The structure of **55** is confirmed by comparing its ^{1}H NMR spectrum with the values listed in Table 1. The adduct **55** appears to undergo slow epimerization when the reaction mixture is kept at room temperature for more than 3 h before work-up, which then upon hydrolysis gives variable yields of a mixture of **6** and its *erythro* isomer (typically 4:1). Although the reasons for the unusual behavior of this reaction are still undetermined, it may be due to the acidity and sensitive nature of the (*p*-nitrophenyl)acetaldehyde moiety. Nevertheless, quenching the reaction at -78 °C or at room temperature within half an hour provides **55** as a pure isomer (40-45%).

An improved method for the generation of adduct **55** was introduced by Dr C. Lowe in our research group (Scheme 14).[38] Schoellkopf and co-workers documented the use of chloro[tris(dimethylamino)]titanium for the enhancement of *threo* vs. *erythro* diastereoselectivity in aldol condensations of bis-lactim ether enolates.[166,167] In an

Scheme 14.



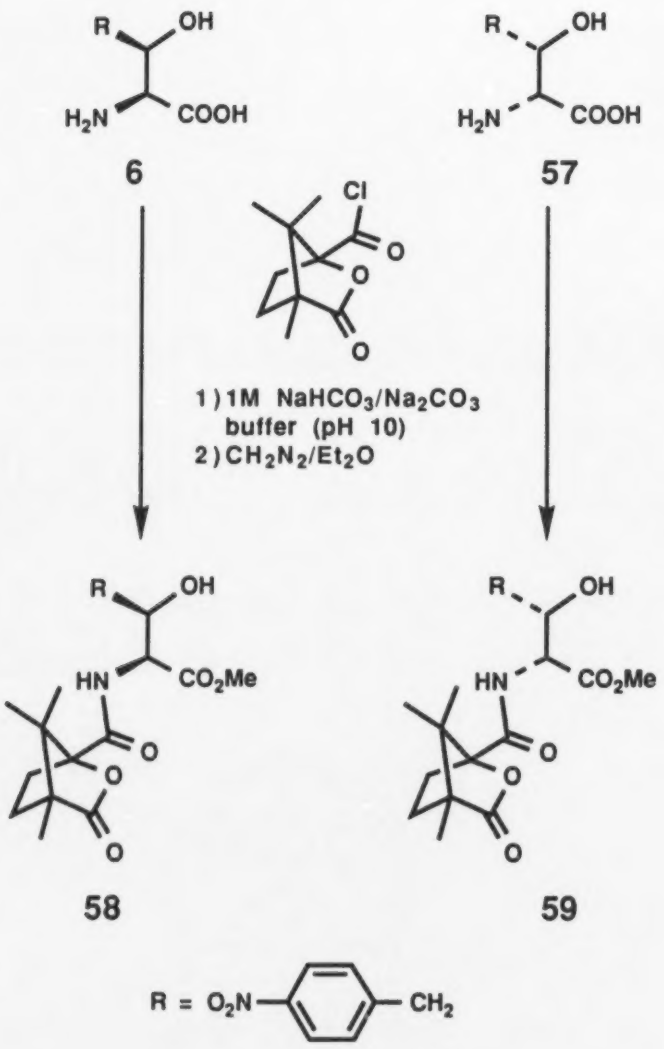
14
14a (enantiomer)

55
56 (enantiomer)

6
57 (enantiomer)

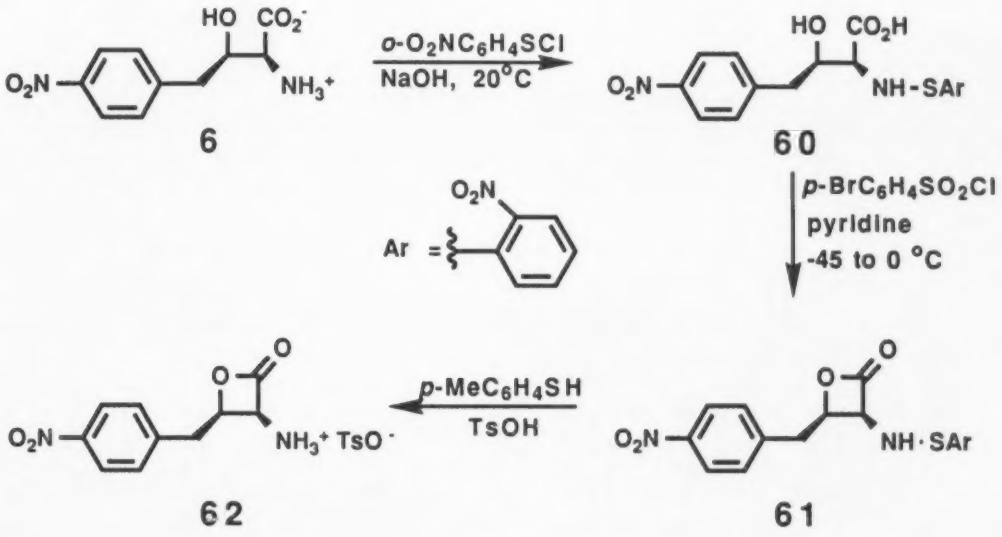
analogous procedure, treatment of the lithium enolate of **14** with chloro-[tris(diethylamino)]titanium[162] presumably gives a titanium enolate. Slow addition of (*p*-nitrophenyl)acetaldehyde **53** to the reaction mixture at -100 °C, with subsequent warming to -78 °C and quenching with aqueous ammonium chloride solution, increases the yield of the aldol reaction to 61% and produces only the *threo* isomer within detection limits. Acidic hydrolysis of the condensation product **55** (61%) and purification by ion exchange chromatography (AG 50W-X8, H^+) affords (*2S,3R*)-2-amino-3-hydroxy-4-(*p*-nitrophenyl)butanoic acid (**6**) in an overall 52% yield from **14** with no detectable trace (by NMR, TLC) of the *erythro* diastereomer. The optical purity of **6** was verified (Scheme 15) by preparation of the methyl ester of its (*S*)-camphanamide derivative **58** and comparison of the spectral data with a similar derivative **59** of (*2R,3S*)-2-amino-3-hydroxy-4-(*p*-nitrophenyl)butanoic acid (**57**). The latter was prepared analogously to **6** from commercially available (*R*)-1-benzoyl-2-(*tert*-butyl)-3-methyl-4-imidazolidinone (**14a**) through the aldol adduct **56** (Scheme 14). The ^1H NMR spectra of the diastereomeric (*S*)-camphanamide methyl esters **58** and **59** are easily distinguishable and show that within detection limits (ca 1%) each derivatized product contains only one optical isomer.

Scheme 15.


R
OH
H2N
COOH
6
57
Cl
O
1) 1M NaHCO3/Na2CO3
buffer (pH 10)
2) CH2N2/Et2O
HN
CO2Me
58
59
R = O2N
CH2

Upon obtaining the amino acid **6**, the syntheses of the β-lactone derivatives follow the methodology devised in Part 1 (Scheme 16).[37] Protection of **6** with (*o*-nitrophenyl)sulfenyl chloride[35,37] forms **60** (90% yield) which is purified by flash

Scheme 16.

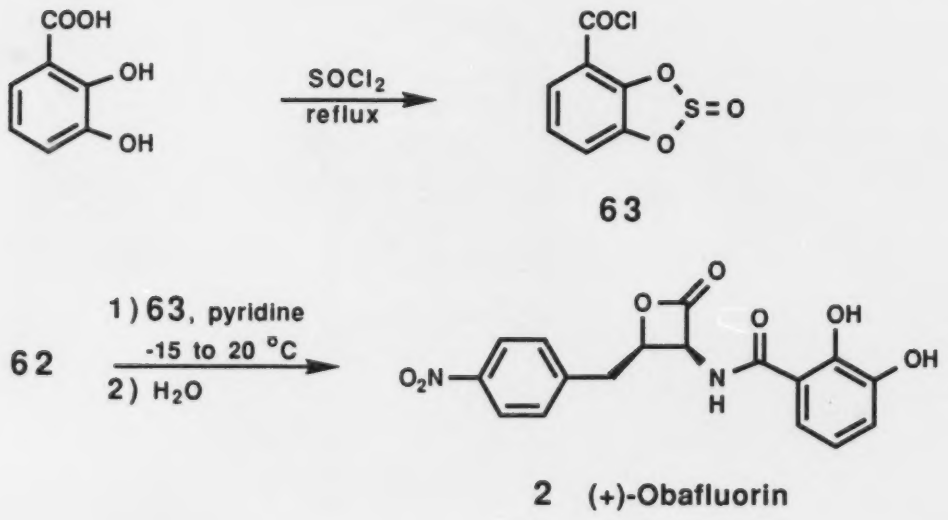


chromatography (1% HOAc/EtOAc). The purification of the protected amino acid on a small scale (< 300 mg) by chromatography appears to be easier and more effective than the recrystallization procedure used for threonine derivatives.[37] Compound **60** is cyclized via carboxyl group activation with 4-bromobenzenesulfonyl chloride in pyridine[36-38] to give the *N*-protected β-lactone **61** (24%). The yield of this step is disappointingly low, but **61** is the only material easily isolable by standard chromatographic purification; the rest of the reaction mixture consists of very polar side products. Work described in Part 1 demonstrates that removal of nitrogen protecting groups from α-amino-β-lactones under carefully controlled acidic conditions provides

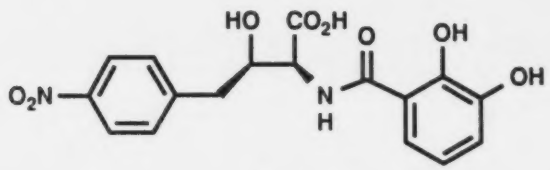
the corresponding salts, which can be subsequently acylated.[37] Thus treatment of **61** with *p*-thiocresol and *p*-toluenesulfonic acid[35,37] generates the stable tosylate salt **62** (93%) of the parent oxetanone.

With the β-lactone tosylate salt **62** available, the next task was preparation of an appropriate acylating reagent to generate obafluorin (**2**). A good acylating reagent for this reaction should have a suitable protecting group(s) for the two phenolic hydroxyl groups which, after the acylation, can be easily deprotected without affecting the sensitive β-lactone functionality. Initial attempts were directed towards the selective protection of the two hydroxyl group with silyl (e.g., diisopropylsilyl) groups,[168,169] but this proved difficult because of the interference from the adjacent carboxyl group. However, the acid chloride **63**, used in the total synthesis of enterobactin (**8**) by Corey *et al.*,[170] is available (Scheme 17) in a single step by reaction of 2,3-dihydroxybenzoic

Scheme 17.



acid with thionyl chloride.[170] It proved to be an ideal acylating reagent for the present transformation. Thus, acylation[37] of the tosylate salt **62** with **63** followed by aqueous work-up, which readily hydrolyzes the cyclic sulfite moiety, produces optically pure (+)-obafluorin (**2**) (57% yield after reverse phase HPLC purification). As expected,[8] obafluorin (**2**) decomposes upon standing in aqueous acetonitrile to the hydrolyzed product **64**. The partly decomposed material can be repurified by rapid HPLC (isocratic

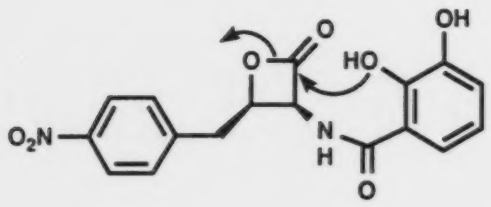


64

elution, 55% acetonitrile-water) to give pure obafluorin ($[\alpha]_D$ +43°, (c = 0.03, MeCN)), which can be stored dry satisfactorily for some weeks at -15 °C under an inert atmosphere without significant decomposition. The optical rotation differs from the literature value[8] ($[\alpha]_D$ +116°, (c = 0.1, MeCN)), possibly because of experimental error in measurement of the rotation at low concentration. To confirm this, the hydrolysis product **64** was further cleaved under acidic conditions to release the free amino acid **6**. Purification by ion exchange chromatography, derivatization to the (*S*)-camphanamide methyl ester as described above for **6** and **57**, and analysis of the ^{1}H NMR spectrum reveals signals corresponding solely to the derivative of **6**, thereby verifying the optical integrity of **2**. The remaining spectral data for **2** agree with published values,[8] and the compound displays potent antibacterial activity against *Staphylococcus aureus* strains in preliminary microbiological tests.

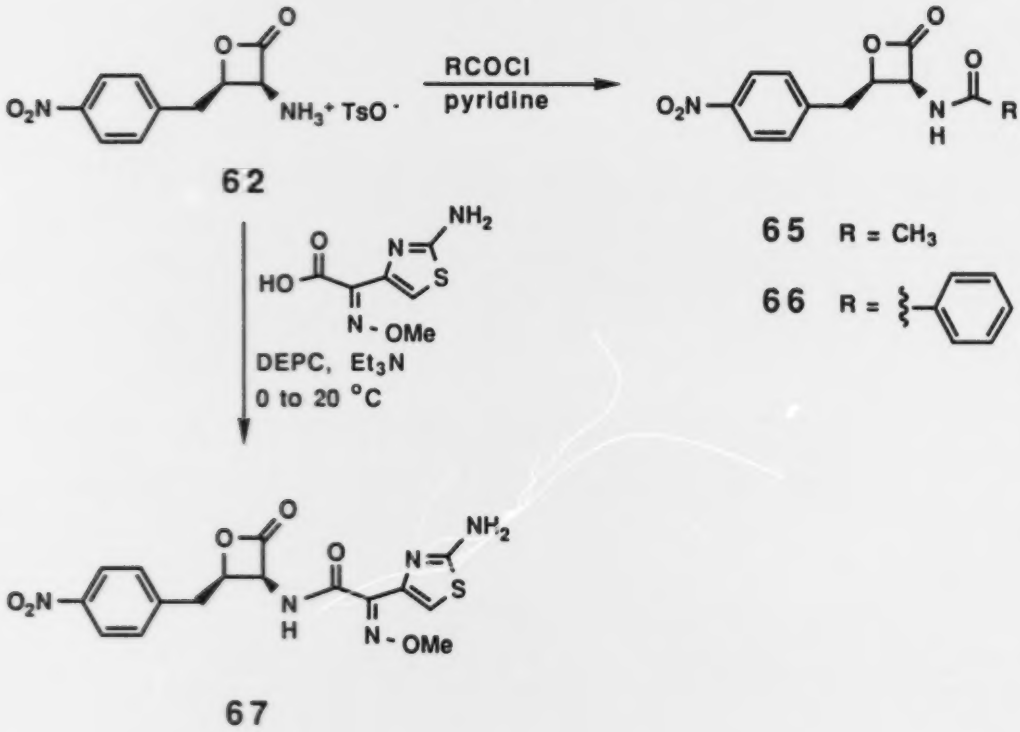
Since the *N*-acyl α-amino β-lactone compounds prepared in Part 1 display very good stability in the absence of nucleophiles, it appears likely that the instability of obafluorin stems from the presence of two free phenolic hydroxyl groups in the vicinity of the β-lactone functionality. One possibility is that the *ortho*-hydroxyl group intramolecularly attacks the β-lactone carbonyl group through a seven-membered transition state to open the lactone (Scheme 18).

Scheme 18.

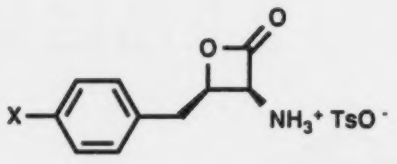


To test whether this acyl group is essential for antibiotic activity, three obafluorin analogs in which the acyl groups are acetyl (**65**), benzoyl (**66**), and 2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl (ATMO) (**67**) were prepared (Scheme 19). Compounds **65** (61% yield) and **66** (81% yield) are obtained by the reactions of the tosylate salt **62** with the corresponding acid chlorides,[37] and **67** is synthesized by a peptide-coupling type of reaction[171] in which the commercially available ATMO-acid is first activated by diethylphosphoryl cyanide (DEPC) and then attached to the tosylate **62** in the presence of triethylamine. Preliminary tests for the biological activities of **65**, **66**, and **67** against several bacterial strains revealed that all three compounds are devoid of antibacterial activity (see Part 4 for the biological assays).

Scheme 19.



In order to understand the role of the unusual aromatic nitro group in the biological activity of obafluorin,[8,9,15] three analogs of the tosylate salt **62** bearing different *para*-substitutents **68** (X = H), **69** (X = Cl), and **70** (X = MeO) were synthesized. Similar substitutions were used to develop quantitative structure-activity relationships for chloramphenicol.[172] The three substituents were selected to test if the nitro group or its electron-withdrawing nature are essential. Although it is also possible that the nitro group works as a masked amino group,[9,15] the amino substituted derivative was not synthesized because such a compound would probably be unstable under physiological conditions due to the presence of an amino group and a β-lactone functionality in the same molecule.

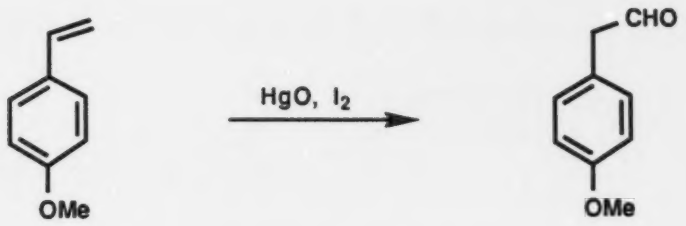


68 X = H
69 X = Cl
70 X = OMe

As in the synthesis of obafluorin, the key intermediates in the syntheses of **68**, **69**, and **70** are the corresponding β-hydroxy α-amino acids. They are accessible by aldol condensation of enolate of the imidazolidinone **14** with the corresponding aldehydes. Phenylacetaldehyde is commercially available and the chloro derivative **54** has been obtained from *p*-chlorostyrene (Scheme 13). The attempted preparation of (*p*-methoxyphenyl)acetaldehyde **71** by reacting *p*-methoxystyrene with lead tetraacetate in TFA[164] gave a black mixture with no detectable aldehyde formation, presumably because the electron-donating ability of the methoxy group renders the *para* vinyl group very reactive and susceptible to polymerization.[164] Oxidation of 2-(*p*-methoxyphenyl)ethanol by alternative methods, such as Swern oxidation,[162] PCC,[173]

CrO_3/DMSO,[174] and *n*-Pr_4NRuO_4 with 4-methylmorpholine *N*-oxide[163] failed to give the desired (*p*-methoxyphenyl)acetaldehyde. However, treatment of *p*-methoxystyrene with mercuric oxide and iodine in a mixed solvent (ether/water) according to a literature procedure affords the aldehyde **71** in quantitative yield (Scheme 20).[175]

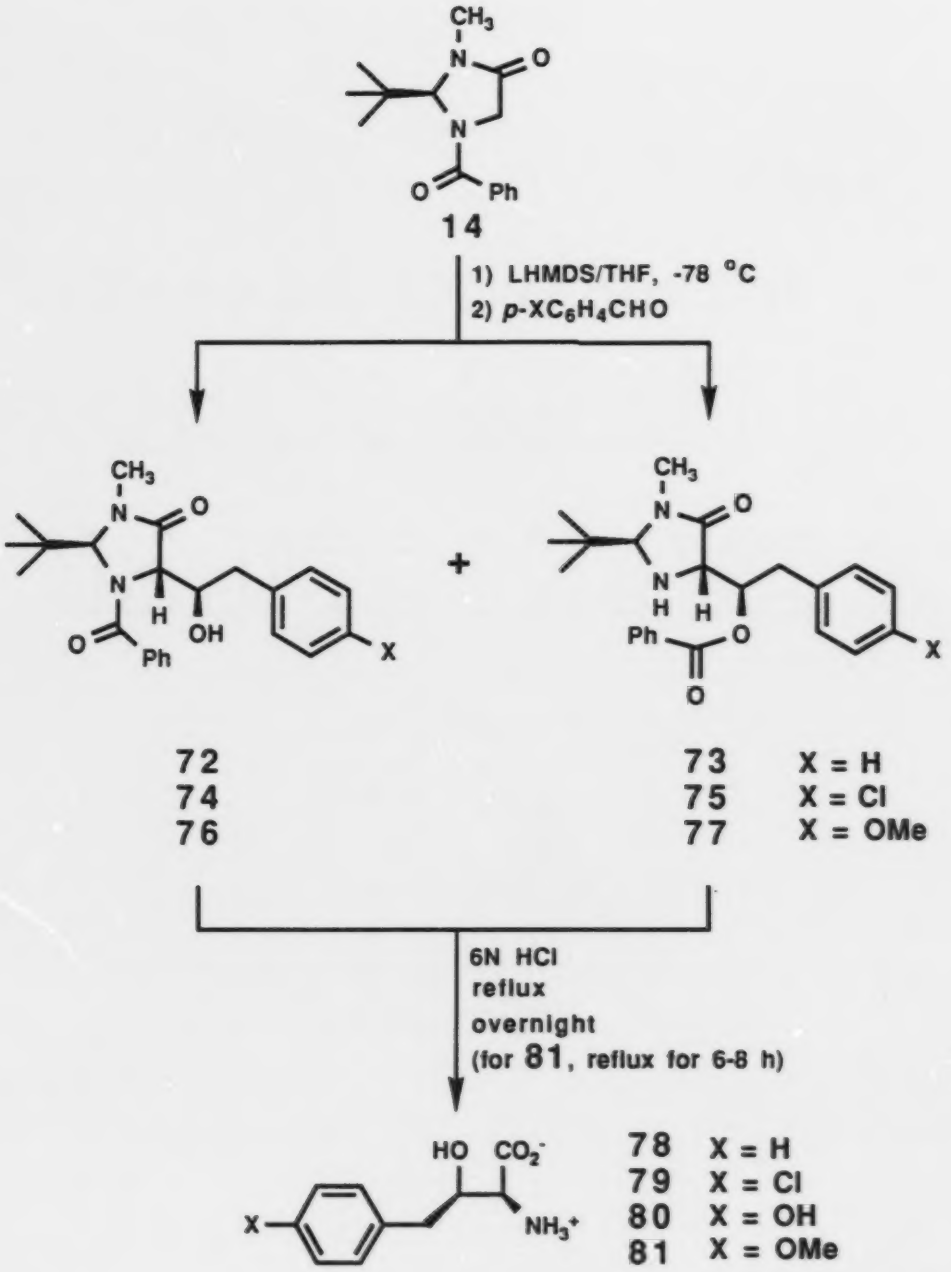
Scheme 20.



71

The *para*-substituted phenylacetaldehydes condense with the enolate of **14** at -78 °C, and in all three cases (X = H, Cl, and MeO), a mixture of normal aldol adduct (**72**, **74**, and **76**) and the rearranged product (**73**, **75**, and **77**) is obtained [X = H, 55% yield, **72/73** (60 : 40); X = Cl, 57% yield, **74/75** (55 : 45); X = MeO, 41% yield, **76/77** (72 : 28)] (Scheme 21). The structures of the isomers were determined by comparing their ^1H NMR spectra with the values listed in Table 1. Interestingly, a solid sample of the mixture **76** and **77** (72 : 28, X = MeO) stored at room temperature changes to the single, more stable isomer **76** in 6 months, whereas no change is found in a mixture of **74** and **75** (55 : 45, X = Cl) under the same conditions. The mixtures of condensation adducts **72/73** and **74/75** are hydrolyzed in a refluxing 6 N HCl solution overnight to give the corresponding β-hydroxy α-amino acids **78** (X = H, 78%) and **79** (X = Cl, 64%), respectively. Similar treatment of the methoxy compounds **76/77** produces the *para*-hydroxy amino acid **80** (quantitative) because of concurrent cleavage of the methoxy substituent. Careful examination of this process

Scheme 21.

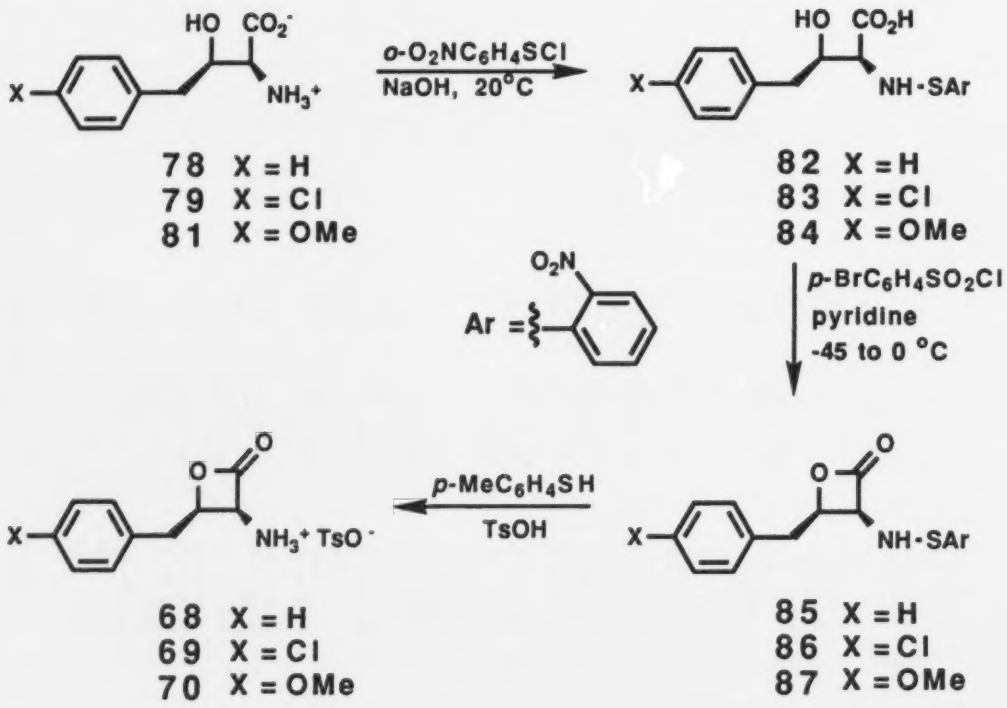

14
1) LHMDS/THF, -78 °C
2) p-XC6H4CHO
72
74
76
73 X = H
75 X = Cl
77 X = OMe
6N HCl
reflux
overnight
(for 81, reflux for 6-8 h)
78 X = H
79 X = Cl
80 X = OH
81 X = OMe

shows that after 6 h the hydrolysis mixture contains mostly methoxy derivative **81** (68%) and that substantial formation of the hydroxy derivative **80** starts after 8 h at reflux. Purification of amino acids **78-81** by ion-exchange chromatography on AG 50W-X8 (H^+ form) resin gives white solids.

The syntheses of the β-lactone derivatives from the corresponding β-hydroxy α-amino acids follow the strategy described in Part 1.[37] Thus, treatment of amino acids **78**, **79**, and **81** with (*o*-nitrophenyl)sulfenyl chloride provides the protected derivatives **82** (70%), **83** (59%), and **84** (45%), respectively. These are subsequently cyclized via carboxyl group activation with 4-bromobenzenesulfonyl chloride in pyridine to the corresponding β-lactones **85** (41%), **86** (25%), and **87** (34%), respectively (Scheme 22).[37,38] Deprotection as described previously with *p*-thiocresol affords the β-lactone tosylates **68** (91%), **69** (100%), and **70** (84%), respectively.[37,38] Studies on the reactions of these three tosylates with different acylating reagents (e.g., acid chloride **63**) to produce obafluorin analogs are currently in progress and are likely to proceed smoothly.

The stability of β-lactone tosylate **70** (X = MeO) can be studied by quantitative solution IR spectroscopy. The β-lactone absorption at 1843 cm^{-1} was monitored for a 1.41 x 10^{-2} M solution of **70** in a mixed solvent [THF/water (3 : 7)]. The $t_{1/2}$ for decomposition of the β-lactone functionality is estimated to be 2-2.5 h, which is similar to that of the corresponding serine derivative.[176] The molecular absorption coefficient ε is calculated to give a value of 364 (cm^2/mol) at 1843 cm^{-1} based on Beer's law $\mathbf{A = lg(I_0/I_t) = \varepsilon lc}$ (A, absorbance; l, length of the cell; c, molar concentration).[177]

Scheme 22.

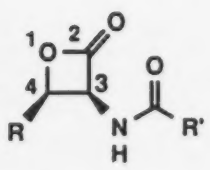


In summary, the first total synthesis of optically pure (+)-obafluorin (**2**) is accomplished in 7% total yield over seven steps through a key β-hydroxy α-amino acid intermediate **6**. The amino acid intermediate is prepared stereospecifically by an aldol condensation between the imidazolidinone (**14**) enolate and (*p*-nitrophenyl)acetaldehyde (**53**) followed by acidic hydrolysis of the aldol adduct **55**. The syntheses of the β-lactone derivatives from amino acid **6** follow the strategy described in Part 1. *N*-Acetyl (**65**), *N*-benzoyl (**66**) and *N*-ATMO (**67**) analogs of obafluorin (**2**) were also prepared. Three β-lactone tosylate salts bearing a *para*-substituted [X = H (**68**), Cl (**69**), and MeO (**70**)] benzyl group at the β-position were synthesized analogously via the corresponding amino acid intermediates **78**, **79**, and **81**.

Part 3. Design and Syntheses of New *N*-Acyl α-Amino β-Lactones

Although the central β-lactone ring is undoubtedly essential for the biological activity of β-lactone antibiotics,[8,9,15] the alkyl side chain (R) attached to C-4 and the acyl group (R'CO) on nitrogen may also play critical roles, as observed for the β-lactam antibiotics.[178] However, exactly how the side chains affect the antibacterial activity of the β-lactone antibiotics is not clear, and one goal of this project was to explore the structure-activity relationships of β-lactone antibiotics and attempt to develop more potent antibacterial compounds. The work described in Parts 1 and 2 provided α-amino β-lactones bearing different alkyl side chains.[37,38] (Figure 15). Examination of a series of these β-lactones (e.g., R'CO = 2,3-dihydroxybenzoyl) would provide information on how structural changes in the alkyl group affect

Figure 15. α-Amino-β-lactones bearing different alkyl groups.



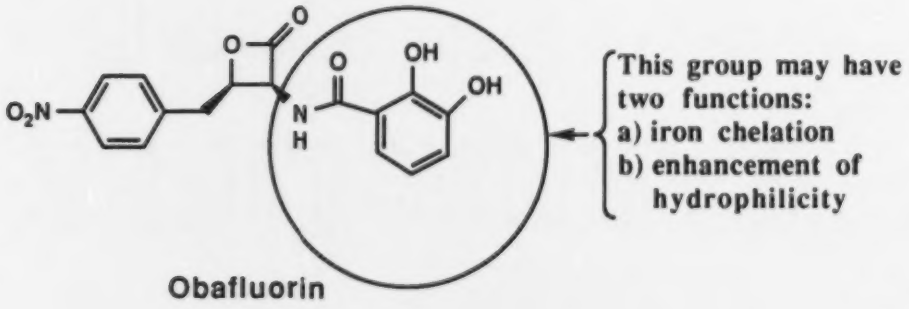
R = CH_3 (and L-*allo* isomer)

R = X–⟨benzene⟩–CH_2 (X = H, Cl, OMe, or NO_2)

antibiotic activity. However, another key feature is the acyl group attached to nitrogen. In this chapter, the design and syntheses of some acylating reagents and their attachment to L-threonine β-lactone are described.

As mentioned earlier, the 2,3-dihydroxybenzamide moiety in obafluorin is similar to the substructure of the strongest natural siderophore enterobactin (**8**, K_f for ferric ion is 10^{49}).[19-21] The loaded siderophore molecules are recognized by specific

receptors on the outer membranes of bacteria. Several penicillins, cephalosporins, and monobactams bearing catechol and other iron-chelating groups are reported to show enhanced activity against certain Gram-negative bacterial strains, presumably because of the improved penetration of the drugs through the outer membranes of bacteria.[22-27] Hence, it may be that the 2,3-dihydroxybenzamide moiety in obafluorin functions in a similar manner and thereby promotes the antibacterial activity of the β-lactone antibiotic. Another possibility is that the obafluorin acyl group may function as a

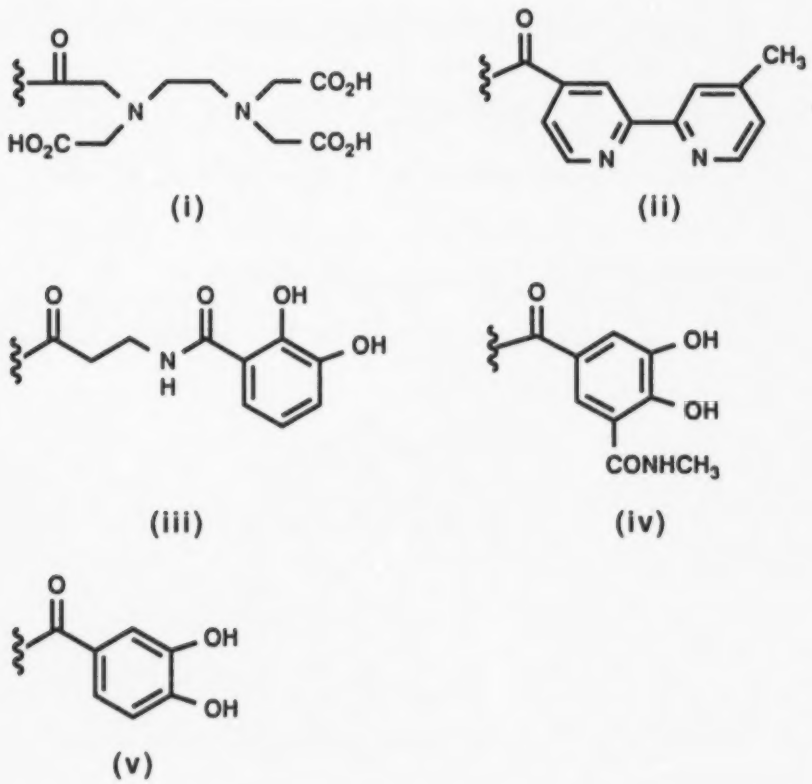


Obafluorin

hydrophilic moiety, which increases solubility and hence bioavailability. Many of the smaller antibiotics penetrate the outer membrane of Gram-negative bacteria by diffusion through 'channels' created by outer membrane proteins known as porins which provide hydrophilic pores for small, water-soluble molecules.[19,178] The presence of hydrophilic groups on an antibiotic molecule usually increases its penetrability and thus the activity. For instance, benzyl penicillin (penicillin G) is not particularly active against Gram-negative bacteria due to inability to penetrate the outer membrane; however, simple substitution on the α-carbon of the hydrophobic acyl side chain with an amino (ampicillin) or carboxyl (carbenicillin) group increases the activity drastically.[178]

Based on the 'iron chelate' hypothesis, five acyl groups were selected as replacements for 2,3-dihydroxybenzoyl group. The first is an ethylenediamine-tetraacetic acid (EDTA) moiety (i). EDTA is a strong iron chelator (K_f is ca 10^{25} for Fe^{3+})[179] and there has been considerable recent research directed toward the design

Scheme 23.

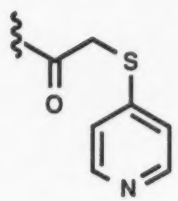


and synthesis of protein-binding and nucleic acid-binding molecules bearing an appended EDTA ligand.[180-184] Attachment of EDTA to the proteins or nucleic acid-binding molecules generates a class of compounds capable of affinity cleavage of their protein or DNA target. It appeared interesting to examine the biological activity of the combination of EDTA and a β-lactone. The second choice is a bipyridine derivative (ii),

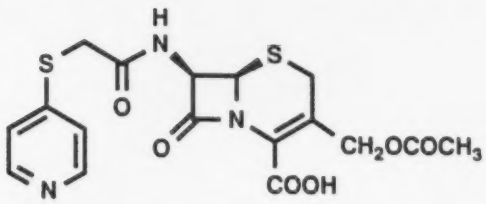
which is also a strong iron chelator (K_f of 10^{17}).[185] In several recent publications,[186,187] the bipyridyl moiety was coupled to a peptide to produce a "model protein", and as with EDTA, this group has been used as a metal chelator for the "chemical nucleases".[188] The other two acyl groups (iii) and (iv) both retain the 2,3-dihydroxybenzamide functionality which possesses strong iron-chelating ability. For example, the monomer of enterobactin, *N*-(2,3-dihydroxybenzoyl)-L-serine, has a K_f of 10^{36}.[20] The selection of these two substituents is based on two considerations: the 2,3-dihydroxybenzamide moiety is maintained, and the structures are such that the aromatic hydroxyl groups are unlikely to affect the stability of the β-lactone moiety by intramolecular nucleophilic attack (Scheme 18). In group (iv), the *ortho*-hydroxyl group is conformationally unfavorable for the intramolecular attack while in structure (iii), the insertion of a β-alanine residue increases the distance between the aromatic hydroxyl groups and the β-lactone carbonyl (separated by nine atoms from *ortho* OH) such that the intramolecular interaction seems unlikely. The fifth choice is the 3,4-dihydroxybenzoyl group (v) which differs from 2,3-dihydroxybenzoyl group only in the substitution positions of the hydroxyl groups. The catechol moiety provides modest iron-chelating ability for the group, and the OH's are located so as to prevent intramolecular nucleophilic attack on the β-lactone.

Based on the 'hydrophilicity' hypothesis, three additional acyl groups were selected to replace the 2,3-dihydroxybenzoyl group (Scheme 24). The first one, a 4-pyridylthioacetyl group (vi), is present in a cephalosporin antibiotic, cephapirin,[189] and several synthetic penicillins and cephalosporins bearing this acyl group exhibit high activities.[190] The pyridine moiety is expected to provide the hydrophilicity for this

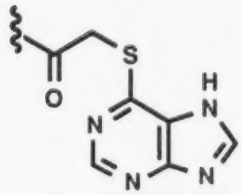
Scheme 24.



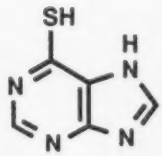
(vi)



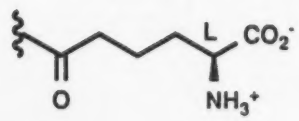
Cephapirin



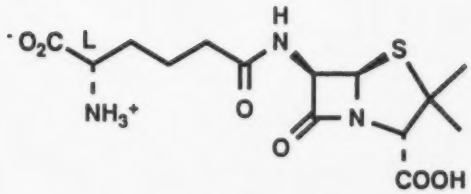
(vii)



6-Mercaptopurine



(viii)

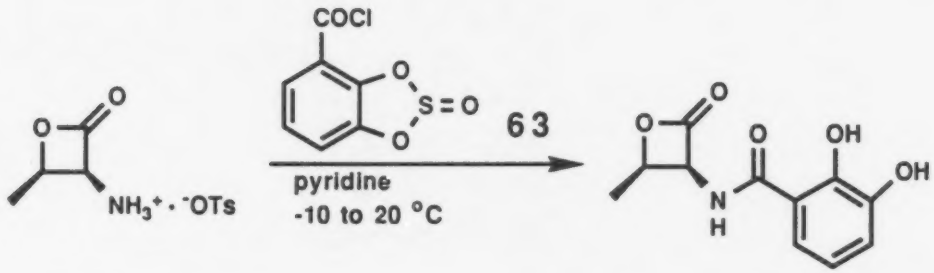


Isopenicillin N

group. The second choice, an analog of the first, is a 6-purinylthioacetyl group (vii). Purine derivatives are hydrophilic[191] and 6-mercaptopurine was one of the first purine derivatives to find application in the treatment of leukemia.[192,193] The nucleophilicity and basicity of these two acyl groups (vi) and (vii) present a challenge to the syntheses as well as the stability of the product β-lactone, but they also provide an opportunity to determine how vulnerable the β-lactone functionality is under these circumstances. The third target acyl group is the L-5-aminoadipyl group (viii), which is present in isopenicillin N.[194] Its terminal amino acid functionality provides hydrophilicity.

Ideally the acyl groups i-viii would be coupled to the nitro substituted β-lactone tosylate **62** because this would provide a more exact comparison with obafluorin. However, **62** is less accessible than threonine β-lactone tosylate **33**, some of whose derivatives (e.g., SQ 26,517(**1**)) are active antibiotics. Hence initial studies focussed on attachment of the chosen acyl groups to **33**. To examine if this approach was reasonable, *N*-2,3-dihydroxybenzoyl L-threonine β-lactone (**88**) was synthesized. Treatment of L-threonine β-lactone tosylate **33** with acid chloride **63** in the presence of pyridine (Scheme 25) affords **88** in 38% yield. In a preliminary test, compound **88** exhibits better activity and with a broader spectrum against several bacterial strains than the natural antibiotic SQ 26,517 (**1**) (see Part 4). Based on this result, it appears reasonable to initially use L-threonine β-lactone as the parent oxetanone nucleus for structure-activity studies on the *N*-acyl group.

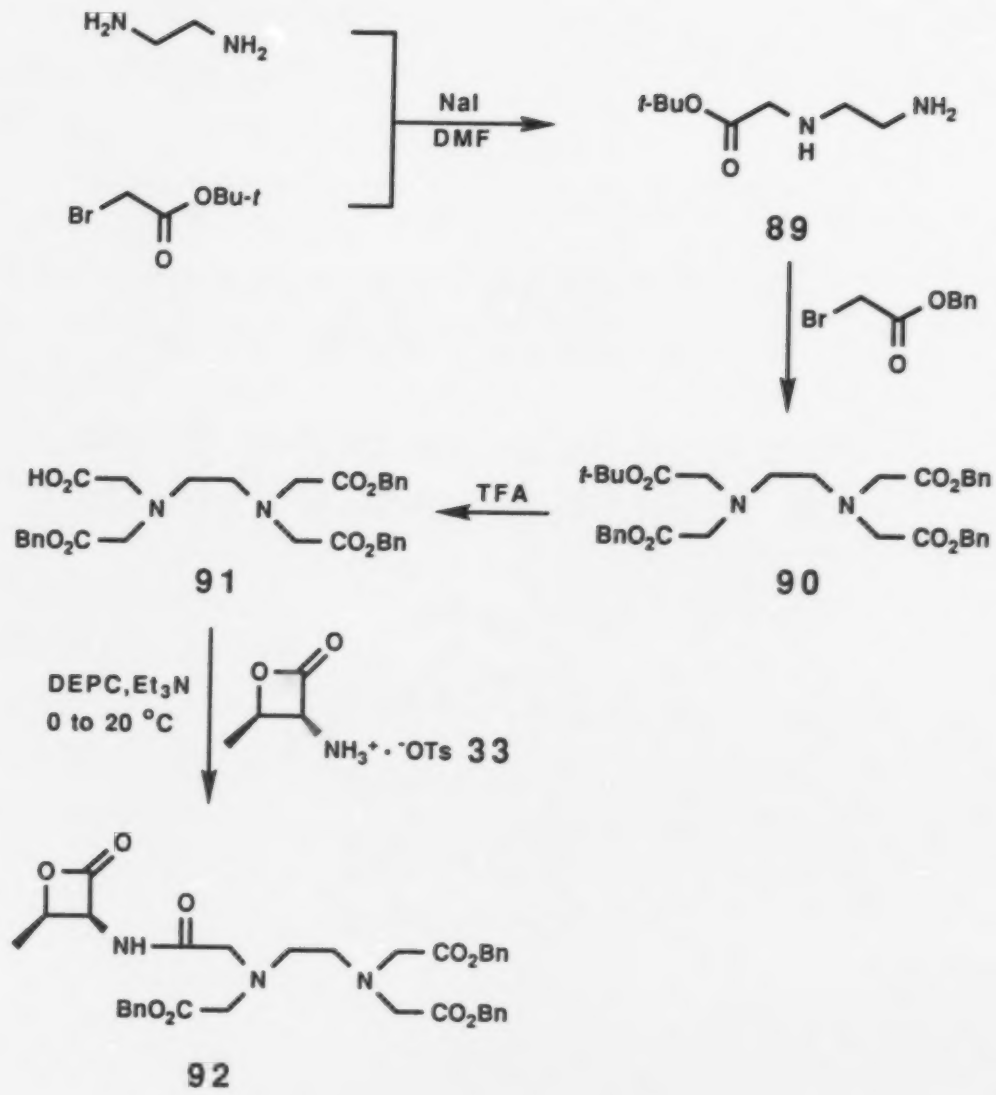
Scheme 25.



33 **88**

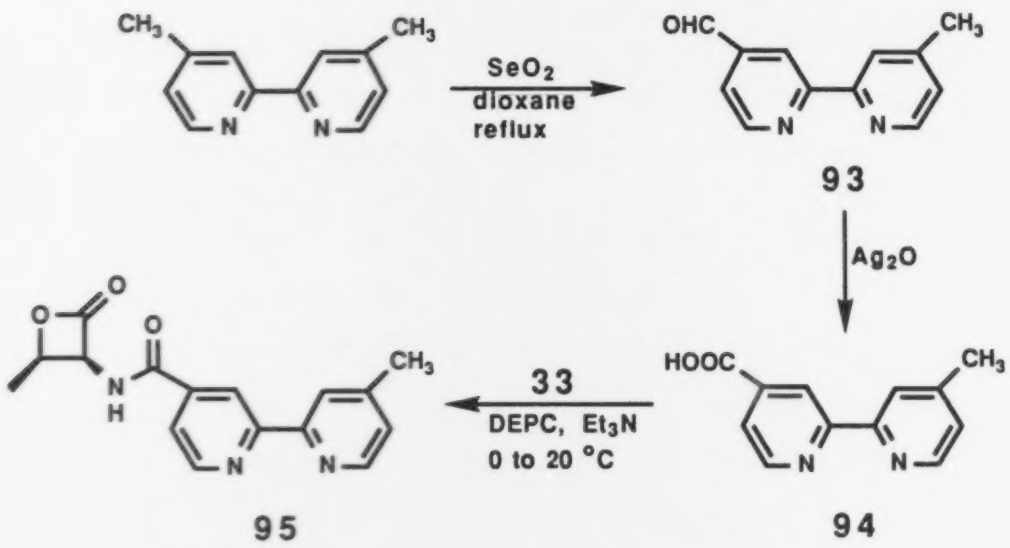
In order to obtain the EDTA-derived acyl group (i), the syntheses of tri-protected EDTA compounds (e.g., EDTA tri-esters) were attempted. The protecting groups must be easily removable under conditions compatible with the β-lactone functionality. Direct esterification of commercially available EDTA (H^+ or Na^+ form) to give either tri- or tetra-esters with the reagents such as 2,2,2-tri-chloroethanol[195-197] or (*p*-bromo)phenacyl bromide[170] appears to be difficult; the reaction stops at either the mono- or di-ester stage as indicated by ^1H NMR spectra and TLC. One possible reason for this is the poor solubility of EDTA in most solvents. Recent syntheses of EDTA tri- or tetra-esters build the EDTA structure from basic starting materials.[180-184] For example, tribenzyl EDTA **91** is prepared in three steps following the procedure outlined in Scheme 26.[180] Commercially available *tert*-butyl bromoacetate reacts with excess ethylenediamine and sodium iodide to provide amine **89** in quantitative yield. Exhaustive alkylation of **89** with benzyl bromoacetate produces the mixed tetra-ester of EDTA **90** (45% yield). Treatment with trifluoroacetic acid converts compound **90** to the EDTA tribenzyl ester **91** in 80% yield. The coupling of EDTA tribenzyl ester **91** to L-threonine β-lactone tosylate salt **33** using diethylphosphoryl cyanide (DEPC) and triethylamine[171] affords the desired oxetanone **92** in 58% yield. The deprotection of **92** potentially can be accomplished under normal hydrogenation conditions,[198] and this study is in progress in our research group.

Scheme 26.



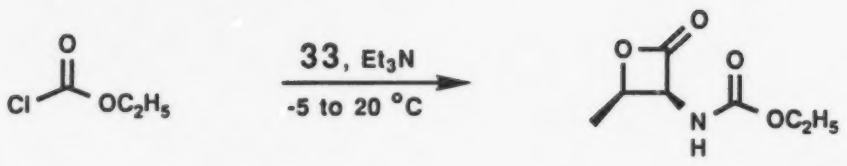
The bipyridyl acid **94** required for acyl group (ii) is available in two steps employing the procedure illustrated in Scheme 27a.[199] Thus, commercially available 4,4'-dimethyl-2,2'-bipyridine is partially oxidized with SeO_2 to the monoaldehyde **93** (21% yield). The corresponding monoacid **94** is obtained by Ag_2O oxidation in 55% yield. Condensation of acid **94** and L-threonine β-lactone tosylate **33** using DEPC in

Scheme 27a.



the presence of Et_3N affords **95** in 61% yield (Scheme 27a). An attempt to couple acid **94** to β-lactone **33** via a mixed anhydride using ethyl chloroformate in CH_2Cl_2 gives **95** as a minor product (5% yield). The major product is the unexpected *N*-(ethoxycarbonyl)-L-threonine β-lactone **96** (34% yield) (Scheme 27b), which probably results from condensation of ethyl chloroformate and the β-lactone tosylate

Scheme 27b.

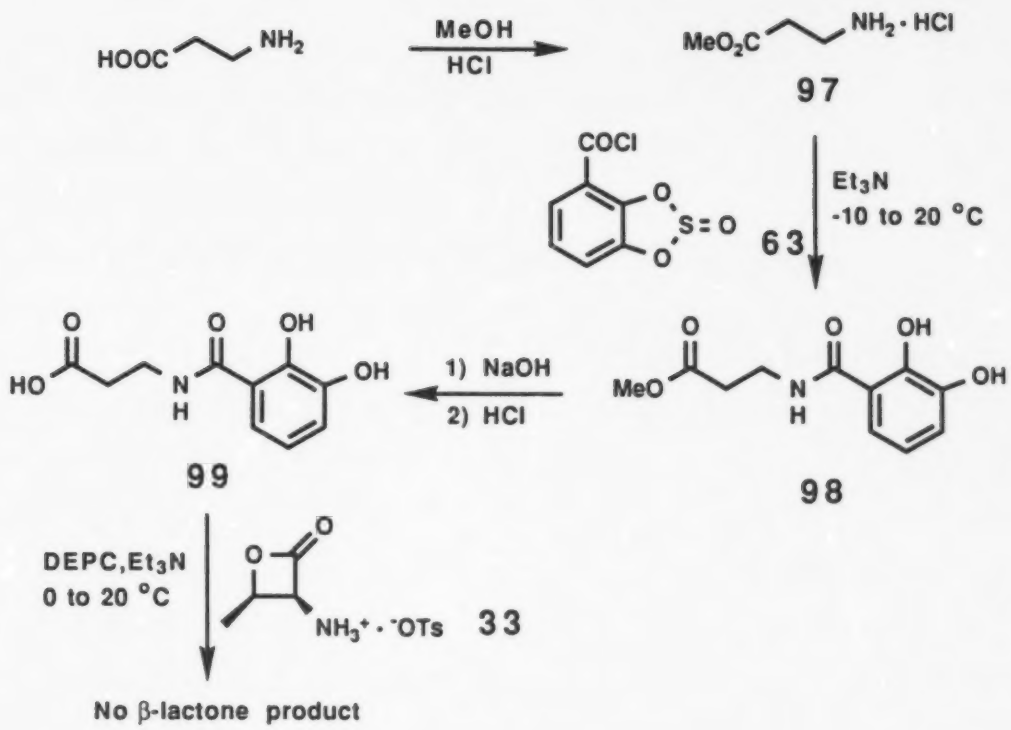


96

salt **33**. This may be due to poor solubility of the bipyridyl acid **94** in CH_2Cl_2. However, use of DMF as the solvent for better solubility gave no β-lactone **95** or **96**, possibly because the basic bipyridine moiety interferes with the mixed anhydride formation in this solvent.

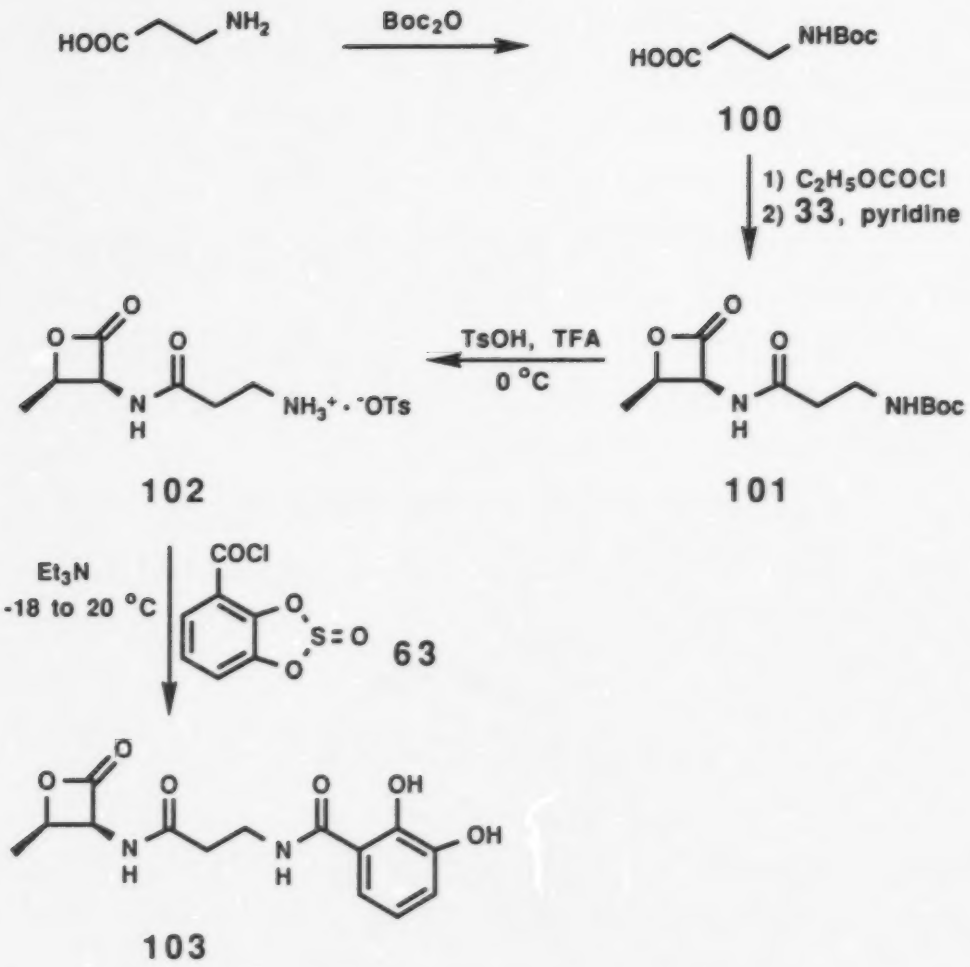
N-(2,3-Dihydroxybenzoyl)-β-alanine **99** was synthesized in 3 steps from β-alanine as the reagent for acyl group (iii) (Scheme 28). Treatment of β-alanine with methanolic HCl gives the methyl ester hydrochloride **97** (84% yield).[200] Reaction of this ester with acid chloride **63** produces *N*-(2,3-dihydroxybenzoyl)-β-alanine methyl ester **98** (55% yield). Subsequent hydrolysis provides the free acid **99** in 70% yield. Attempts to couple this acid to the amino group of L-threonine β-lactone under a variety of conditions fails to produce the desired β-lactone. This problem probably stems from reaction of the two free hydroxyl groups with the activating reagents, thereby hindering the transformation.

Scheme 28.



To overcome this difficulty, an alternative route was devised (Scheme 29). *N-tert*-Butoxycarbonyl (Boc) β-alanine **100** (26%) is available from treatment of β-alanine with di-*tert*-butyl dicarbonate.[201] Formation of the mixed anhydride of **100** with ethyl chloroformate followed by reaction with L-threonine β-lactone tosylate **33** yields the β-lactone **101** in 65% yield. Removal of the Boc group by treatment with trifluoroacetic acid in the presence of *p*-toluenesulfonic acid quantitatively generates the tosylate salt **102**. Acylation of this with acid chloride **63** gives the desired β-lactone **103** in 68% yield (purified by R-18 reverse phase HPLC with 15% acetonitrile/water).

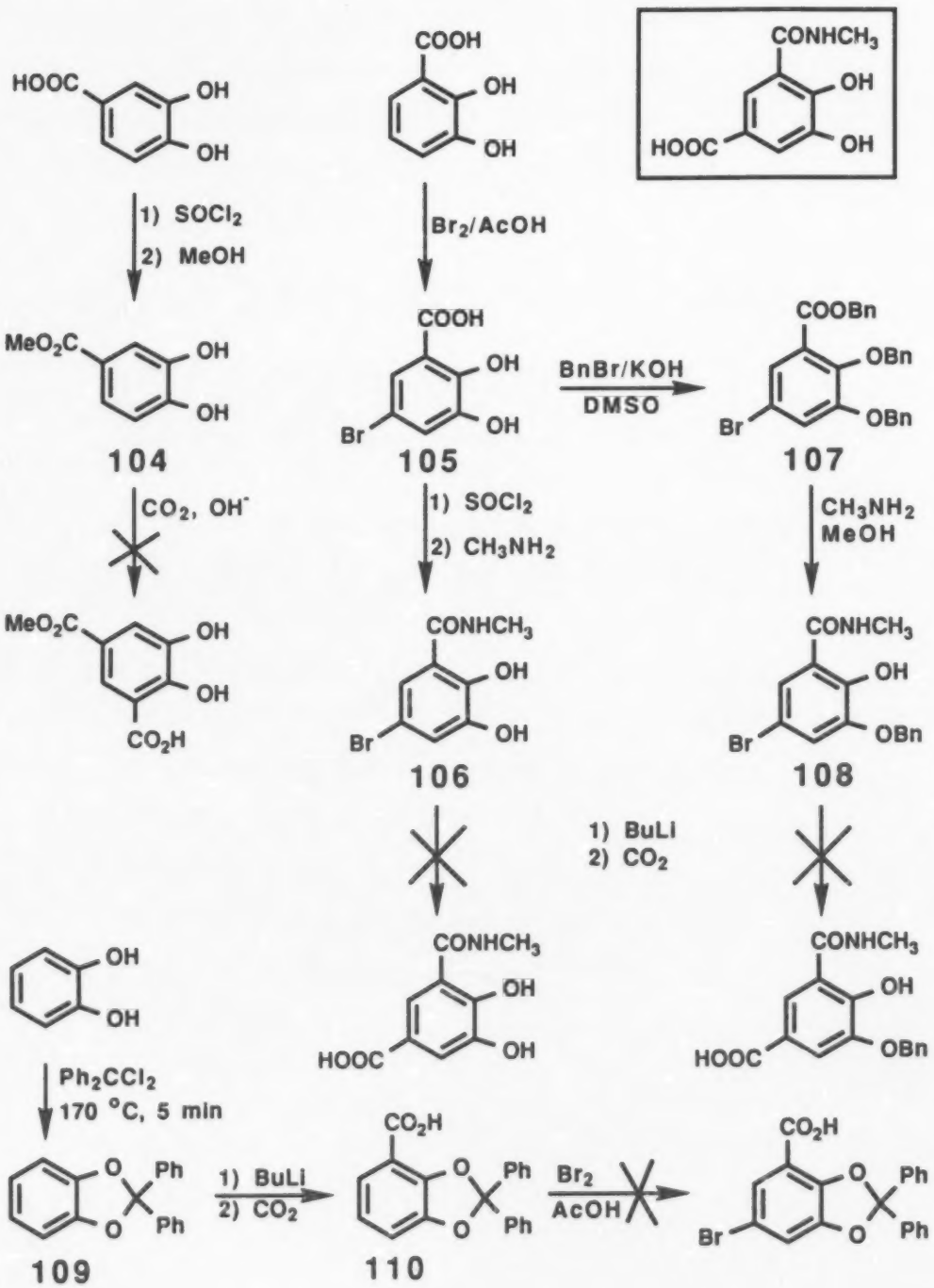
Scheme 29.



Compared with obafluorin (**2**), compound **103** shows good stability; a sample of **103** (pure white solid) is unchanged after being stored at room temperature for a month (checked by ^{1}H NMR).

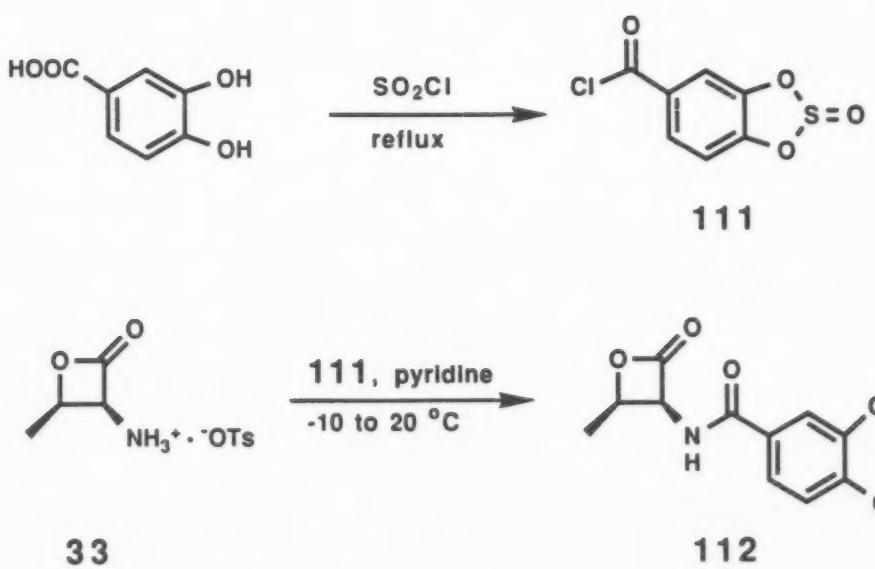
The synthesis of 3-carboxyl-5,6-dihydroxy-*N*-methylbenzamide for acyl group (iv) was attempted through several routes; however, none of them proved successful (Scheme 30). For example, treatment of methyl 3,4-dihydroxybenzoate (**104**), prepared from the acid chloride and methanol (78% yield), with carbon dioxide in the presence of base (Kolbe-Schmidt reaction)[202] results in the recovery of starting material. Another route starts from 2,3-dihydroxybenzoic acid, which is brominated to give bromide **105** (92% yield).[203] Compound **105** is converted to benzamide **106** by reaction of the corresponding acid chloride with methylamine in 48% yield. Reaction of the benzamide **106** with *n*-butyllithium followed by addition of solid carbon dioxide only results in the recovery of the starting benzamide. Protection of the two hydroxyl groups as an acetonide[204] using either acetone or 2,2-dimethoxypropane (DMP) gives a complicated mixture probably because of interference from adjacent methylamido group. Exhaustive benzylation of compound **105** with benzyl bromide gives benzyl ester **107** (70% yield). Treatment of ester **107** with methylamine surprisingly deprotects the adjacent benzyl ether and gives amide **108** (75% yield), and attempts to reprotect **108** with benzylbromide only gives the starting material. Neither bromide **107** nor bromide **108** can be converted to the benzoic acids by sequential reaction with *n*-butyllithium and solid carbon dioxide.[205] A third route starts from acetal **109**, which is prepared by treatment of catechol with dichlorodiphenylmethane (46% yield). Lithiation of **109** with *n*-butyllithium and quenching with solid carbon dioxide gives the acid **110** (36% yield).[205] Bromination of **110** with bromine in acetic acid also simultaneously deprotects the ketal (other common reagents, such as NBS,[206] fail to brominate). Since the β-lactone bearing acyl group (iii) **103** had already been obtained, and the choice of acyl groups (iii) and (iv) are based on similar considerations, the synthesis of acyl group (iv) was not pursued further.

Scheme 30.


HOOC
OH
OH
1) SOCl2
2) MeOH
MeO2C
104
CO2, OH-
MeO2C
CO2H
COOH
Br2/AcOH
Br
105
1) SOCl2
2) CH3NH2
CONHCH3
106
1) BuLI
2) CO2
CONHCH3
HOOC
BnBr/KOH
DMSO
COOBn
OBn
107
CH3NH2
MeOH
CONHCH3
OBn
108
CONHCH3
HOOC
OBn
Ph2CCl2
170 °C, 5 min
O
Ph
109
1) BuLI
2) CO2
CO2H
110
Br2
AcOH
Br

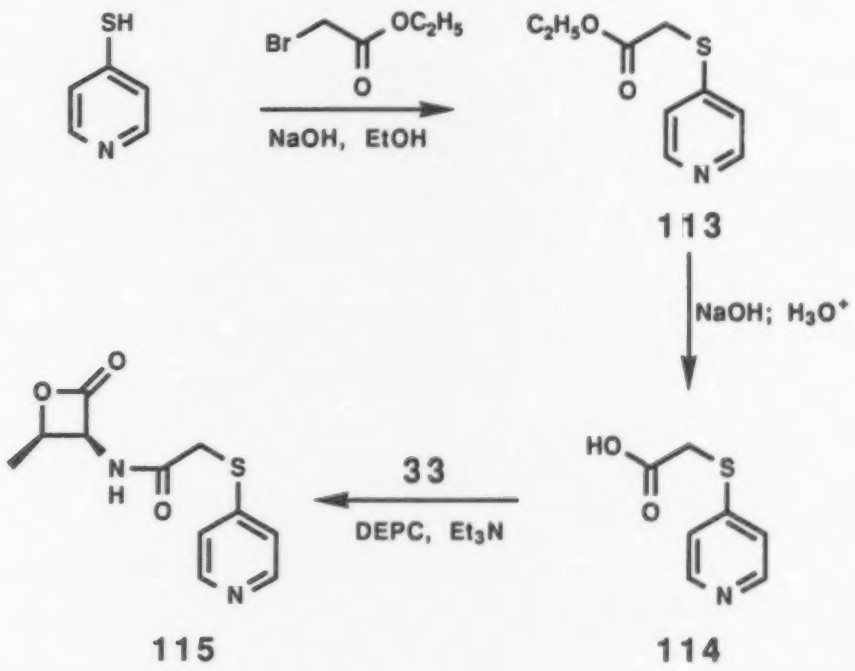
In order to obtain a β-lactone bearing acyl group (v), acid chloride **111** was prepared analogously to **63** by heating 3,4-dihydroxybenzoic acid in refluxing thionyl chloride. Purification of **111** by distillation gives a colorless liquid which solidifies upon storing at -20 °C. Reaction of the tosylate salt **33** and acid chloride **111** in the presence of pyridine produces *N*-3,4-dihydroxybenzoyl L-threonine β-lactone **112** in 90% yield (Scheme 31).

Scheme 31.



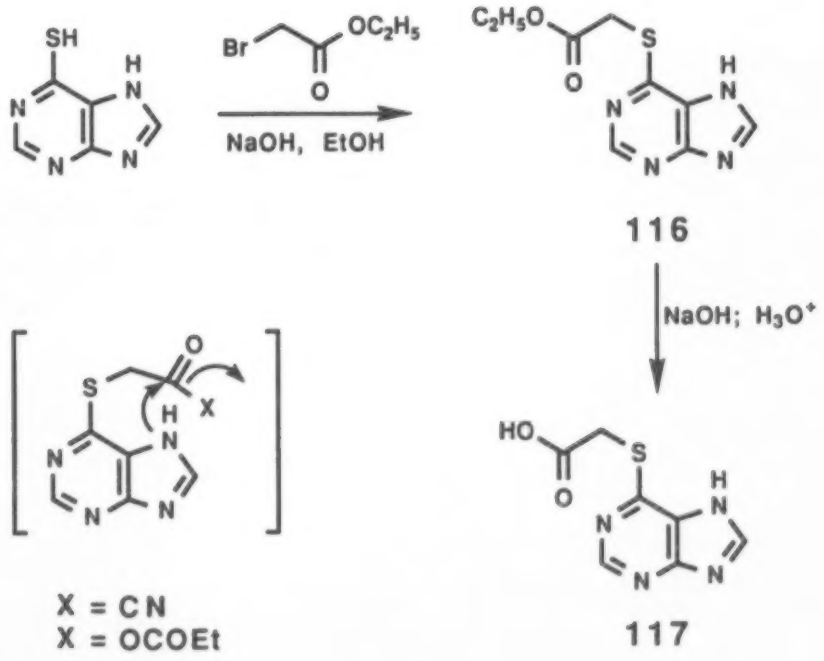
The synthesis of 4-pyridylthioacetic acid **114** was accomplished in two steps (Scheme 32). 4-Mercaptopyridine reacts with ethyl bromoacetate under basic conditions to afford the ester **113** in quantitative yield. Basic hydrolysis of **113** followed by ion exchange purification (AG 50W-X8, H^+) provides **114** in 91% yield. Attempted coupling of acid **114** to L-threonine β-lactone tosylate **33** with ethyl chloroformate does not give the desired β-lactone, probably because the basic and nucleophilic pyridine moiety interferes with the mixed anhydride formation. However, condensation of acid **114** and tosylate **33** using DEPC in the presence of Et_3N produces the desired β-lactone **115** in 76% yield. Unfortunately, this compound is unstable and polymerizes to an insoluble solid at room temperature over several hours. This probably occurs by interaction of the nucleophilic pyridine moiety with the rather vulnerable β-lactone.

Scheme 32.



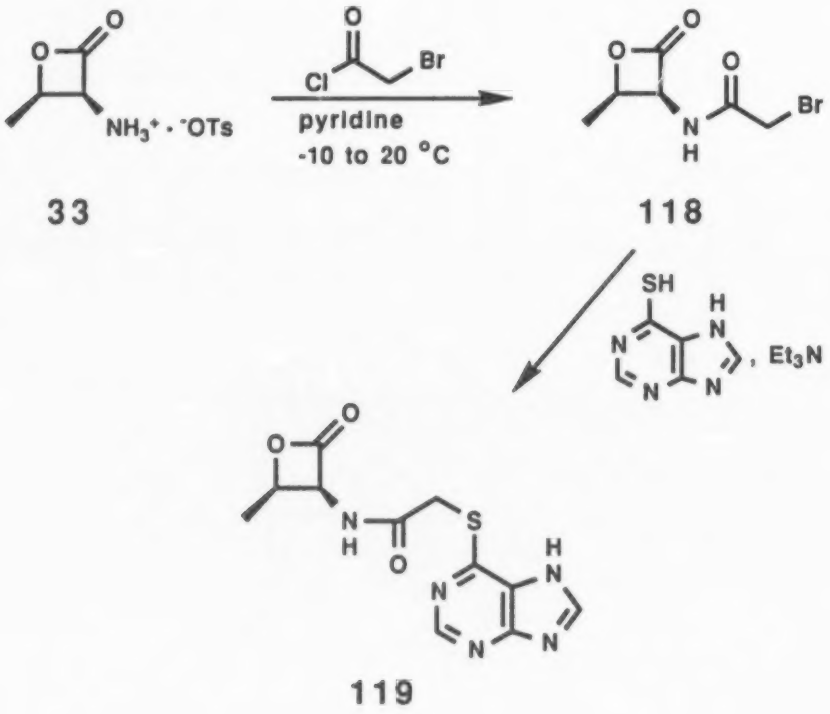
The preparation of 6-purinylthioacetic acid **117** can be achieved in a fashion similar to that used for **113** (Scheme 33). 6-Mecaptopurine reacts with ethyl bromoacetate in the presence of base to give ester **116** (96% yield), which is hydrolyzed to the acid **117** (81% yield). However, neither the mixed anhydride method nor the use of DEPC with Et_3N condenses acid **117** with L-threonine β-lactone tosylate **33**. Presumably, this is because intramolecular attack by the nucleophilic nitrogen of the aromatic purine ring may occur on the activated carbonyl group through a six-membered transition state (Scheme 33). Therefore, an alternative approach was developed (Scheme 34) based on the observations that the bromine of bromoacetates can be easily replaced by thiols and that L-threonine β-lactone is stable in the presence

Scheme 33.



of aromatic thiols used in deprotection.[37,38] Treatment of tosylate **33** with bromoacetyl chloride forms *N*-bromoacetyl L-threonine β-lactone **118** in 95% yield. Reaction of **118** with 6-mercaptopurine monohydrate in the presence of Et_3N provides the β-lactone **119** in 66% yield. In contrast to **115**, compound **119** appears to be very stable.

Scheme 34.

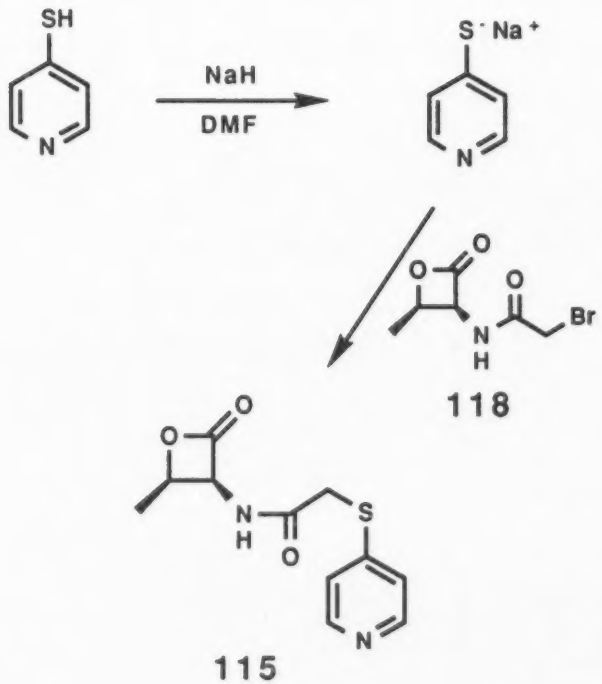


33 **118** **119**

The same approach was attempted for the synthesis of 4-pyridylthioacetyl derivative **115**. However, treatment of *N*-bromoacetyl L-threonine β-lactone (**118**) with 4-mercaptopyridine in the presence of Et_3N gives only very low yields of **115** and

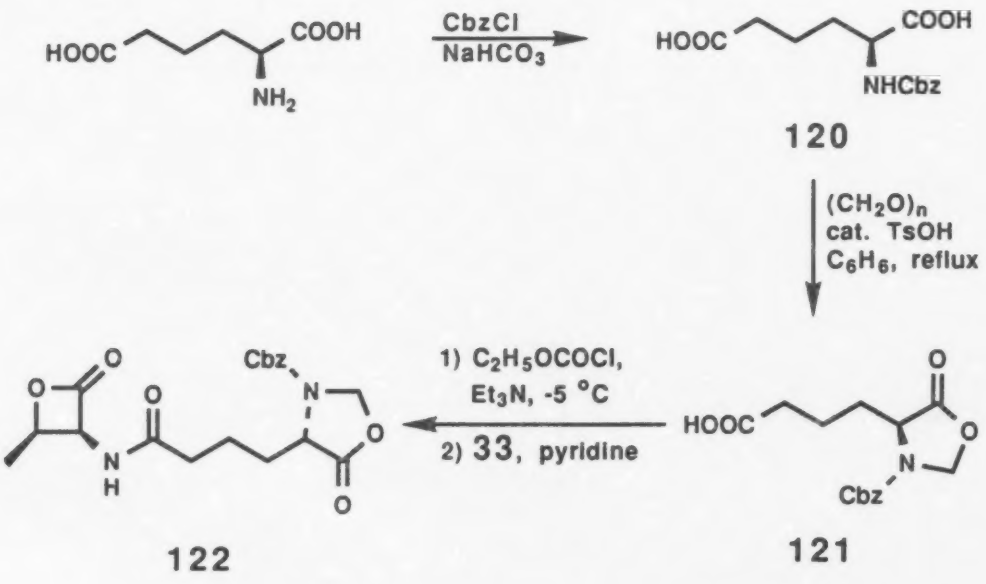
recovery of starting material. This is probably due to the weak nucleophilicity of the thiol group in 6-mercaptopyridine, which exists predominantly in the thione rather than thiol form.[207,208] Since thiolate anions are much stronger nucleophiles than the corresponding thiols, 4-mercaptopyridine was treated with sodium hydride and immediately allowed to react with *N*-bromoacetyl L-threonine β-lactone (**118**). Under these conditions, the β-lactone **115** forms in quantitative yield (Scheme 35). However, as in the previous preparation (Scheme 32), the product lactone is unstable and polymerizes quickly at room temperature.

Scheme 35.



α-L-Aminoadipic acid suitable for acyl group (viii) is commercially available, but to selectively activate one carboxyl group (C-6) is difficult. Therefore, the terminal amino acid group should be protected and this is achieved in two steps, as illustrated in Scheme 36.[209,210] Treatment of α-L-aminoadipic acid with benzyl chloroformate generates *N*-Cbz-α-L-aminoadipic acid **120** (69% yield), which condenses with formaldehyde in the presence of a catalytic amount of *p*-toluenesulfonic acid to provide the oxazolidinone **121** (10% yield).[209,210] The low yield in the second step results from poor efficiency in removing water from the reaction by molecular sieves (4 Å) and

Scheme 36.



the sublimation of solid paraformaldehyde (2 equivalent used). This is typical for small scale reactions, because on a larger scale the water is removed more efficiently using a Dean-Stark apparatus and the small amount of paraformaldehyde sublimation does not affect the yield appreciably.[210] Conversion of the protected α-aminoadipic acid **121** to

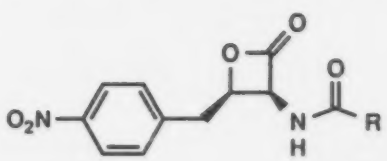
a mixed anhydride with ethyl chloroformate and then coupling to L-threonine β-lactone tosylate **33** in the presence of base produces lactone **122** in 90% yield. Deprotection of the oxazolidinone masked amino acid group can potentially be accomplished by hydrogenation[209] and this work is in progress.

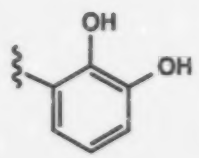
In summary, eight acyl groups were chosen based on two presumed functions of the 2,3-dihydroxybenzoyl group in the β-lactone antibiotic obafluorin (**2**), i.e., iron-chelating and/or hydrophilicity-promoting. The syntheses of seven of the acyl groups as the carboxylic acids proceed readily, as described above. The successful coupling of these acids to L-threonine β-lactone tosylate **33** employs either a mixed anhydride approach or a peptide-coupling (DEPC and Et_3N) strategy. Nucleophilic substitution of *N*-bromoacetyl L-threonine β-lactone **118** with 6-mercatopurine or 4-pyridylsulfide gives the corresponding β-lactone derivatives **119** and **115**. The antibacterial activities of the synthetic *N*-acyl β-lactones are reported below in Part 4.

Part 4. Biological Activities of α-Amino β-Lactones

The synthetic *N*-acylated α-amino β-lactones including SQ 26,517 (**1**), obafluorin (**2**), the *N*-(*o*-nitrophenyl)sulfenyl-protected derivatives, and the deprotected β-lactone tosylate salts were assayed for any antibacterial activity against a number of bacterial strains. The preliminary tests reveal some interesting results which appear promising for the further development of this class of antibacterial agents.

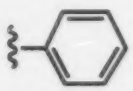
The tested compounds are divided into three groups according to their structures. The first group consists of obafluorin (**2**) and the three analogs bearing different *N*-acyl groups (**65**, **66**, and **67**). Listed below are their structures and the results of the biological test are shown in Table 2.



2 R = 

(Obafluorin)

65 R = CH_3

66 R = 

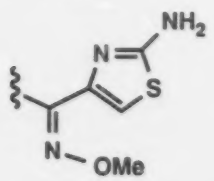
67 R = 

Table 2. Antibacterial activity of obafluorin (**2**) and its analogs bearing different acyl groups.

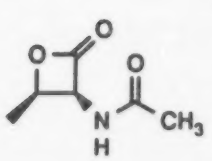
Compound No. / Organisms	Inhibition Concentration (μg/mL)*			
	2	**65**	**66**	**67**
Staph. aureus				
25923	25	X	X	X
13565	50	X	X	X
E. coli				
11229	X	X	X	X
11775	X	X	X	X

X: no inhibition at 200 μg/mL.

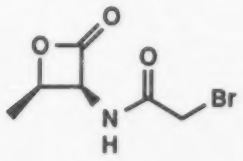
* minimum concentration to give an inhibition zone by agar diffusion method (see Experimental Section).

As shown by the results, only obafluorin (**2**) among the four tested compounds is active against Gram-positive *S. aureus* ATCC 25923 and 13565. The 2,3-dihydroxybenzoyl group may be the key to the difference, but it is difficult to define its role. In Part 3, we have suggested that it may serve two possible functions, i.e. "iron-chelating" and "hydrophilicity-promoting". The ATMO group which has been successful in promoting the activity of the β-lactam antibiotics,[2] is hydrophilic; yet compound **67** bearing this moiety is inactive. Thus, it appears that the function of 2,3-dihydroxybenzamide group is more complicated than simply providing hydrophilicity for the antibiotic. None of the four compounds is active against Gram-negative *E. coli* strains. This may be related to the high degree of susceptibility of obafluorin to hydrolysis by β-lactamases.[8]

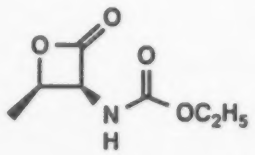
The second group includes 10 L-threonine β-lactone derivatives. In addition to the *N*-acylated derivatives, i.e., SQ 26,517 (**1**), its bromo-substituted analog **118** and those prepared in Part 3 (**88**, **95**, **96**, **103**, **112** and **119**), *N*-(*o*-nitrophenyl)sulfenyl L-threonine β-lactone (**23**), and the deprotected tosylate salt **33** were also tested for their antibacterial activities; the results are listed in Table 3.



1



118



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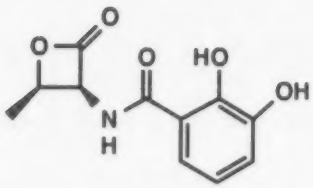

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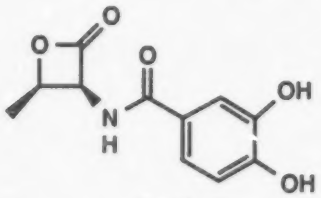

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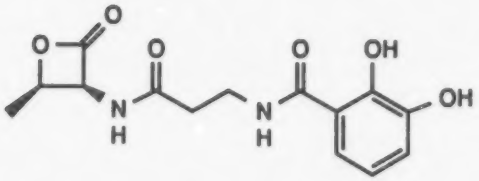

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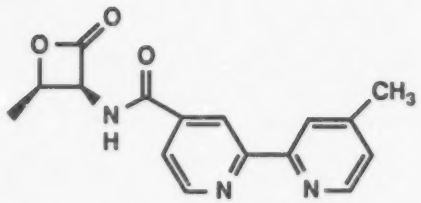
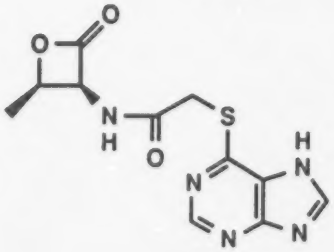


95



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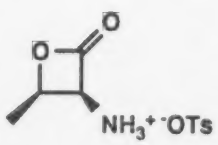
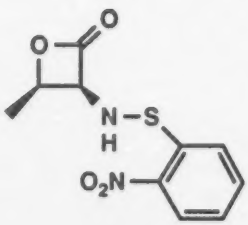


33



23

Table 3. Antibacterial activity of L-threonine β-lactone derivatives.

Organisms \ Compound No.	Inhibition Concentration (μg/mL)*									
	1	**118**	**96**	**88**	**112**	**103**	**95**	**119**	**33**	**23**
Staph. aureus 6538	250	250	X	125	X	X	125	125	X	< 62
Strep. faecalis 7080	X	250	X	125	X	X	500	125	X	62
Serratia marcescens 13380	X	500	X	250	X	X	X	X	X	X
K. pneumoniae 11296	X	500	X	250	X	X	X	X	X	X
Proteus vulgaris 13315	X	500	X	500	X	X	X	X	X	X

X: no inhibition at 500 μg/mL.

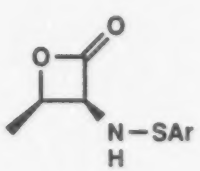
* See Table 2.

Although the natural antibiotic SQ 26,517 (**1**) demonstrates weak activity only against *S. aureus* 6538, the synthetic bromo-substituted derivative **118** is active against all the tested strains, but with weak inhibition. The most potent *N*-acylated L-threonine β-lactone is **88** bearing the 2,3-dihydroxybenzamide moiety. However, neither compound **112**, which differs from **88** only in the substitution positions of the two phenolic hydroxyl groups, nor compound **103**, which has a 2,3-dihydroxybenzamide moiety separated from the β-lactone nucleus by a β-alaninyl moiety, is active against the tested strains. This may suggest that the geometric locations and/or the conformation of the active groups (e.g., the β-lactone and the aromatic hydroxyl groups) are critical for the antibacterial activity; the active site of the particular bacterial enzyme is likely to require a specific geometry and/or conformation of the antibiotic for the inhibition. While the iron-chelating and/or the hydrophilicity-promoting functions of the acyl groups in **88** and **103** may still be effective by assisting the penetration of the

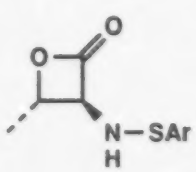
molecules, the specific structural requirement is perhaps decisive for the activity. Both compound **95** bearing a bipyridine type "iron-chelating" acyl group and compound **119** bearing a hydrophilic thiopurine moiety exhibit weak activities against *S. aureus* and *S. faecalis*, which may to some extent support the presumed functions of the acyl side chains. Compound **96** having a urethane group is devoid of activity against all strains. The β-lactone tosylate salt **33** is also inactive, probably due to its short life-time in the bio-environment.

Unexpectedly, the *N*-(*o*-nitrophenyl)sulfenyl L-threonine β-lactone **23** demonstrates extraordinary inhibition (large inhibition zone at 62 μg/mL) against *S. aureus* and *S. faecalis*. This interesting result encouraged us to test a group of *N*-(*o*-nitrophenyl)sulfenyl α-amino β-lactone analogs.

In addition to compound **23**, four other *N*-protected β-lactones **32**, **61**, **86** and **87** prepared in Parts 1 and 2 were tested. A key question was whether (*o*-nitrophenyl)sulfenyl compounds without β-lactone moiety are active under the same test conditions. Therefore, *o*-nitrothiophenol (**37**) and the corresponding disulfide **36** were also tested. The results are shown in Table 4.



23



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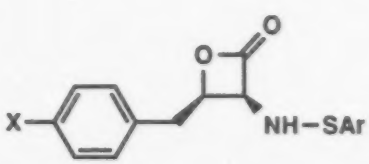
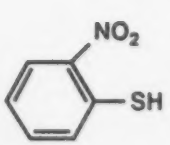


61 X = NO_2
86 X = Cl
87 X = OMe



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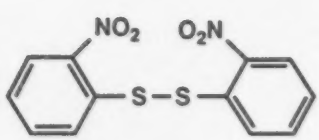

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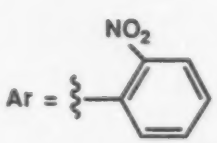

Table 4. Antibacterial activity of *N*-(*o*-nitrophenyl)sulfenyl α-amino β-lactones.

Compound No. / Organism	Inhibition Concentration (μg/mL)*						
	23	**32**	**61**	**86**	**87**	**37**	**36**
***Staph. aureus* 6538**	12.5	25	4.0	3.1	1.5	10	X

X: no activity at 100 μg/mL.

* See Table 2.

All the tested *N*-(*o*-nitrophenyl)sulfenyl β-lactones exhibit potent activities against *S. aureus*, particularly the three bearing a *para*-substituted benzyl group at C-4 (**61**, **86**, and **87**). *o*-Nitrothiophenol **37** also shows comparable activity, while the corresponding disulfide **36** is inactive at 100 μg/mL. A literature search indicated that (*o*-nitrophenyl)sulfenamides are known to have antifungal properties, but poor solubility is a drawback of these compounds.[212,213] At this stage, it is not clear whether the potent activity of these *N*-protected β-lactones results only from the (*o*-nitrophenyl)sulfenyl moiety, or whether the β-lactone ring is also critical. To clarify this, an activity test involving the corresponding uncyclized *N*-protected β-hydroxy α-amino acids is in progress. Also, the syntheses of the phenylsulfenyl protected β-lactones are ongoing in our research group; the absence of the *o*-nitro group may diminish the protecting group's influence on the activity, allowing a clearer observation of the significance of the β-lactone functionality.

In conclusion, this research has produced an effective general method for stereospecific syntheses of β-substituted α-amino β-lactones. It led to the first total synthesis of (+)-obafluorin (**2**), the production of SQ 26,517 (**1**), and the generation of

a large number of their analogs. These compounds were tested for antibacterial activity in preliminary fashion, and several synthetic *N*-acyl β-lactones are more active than the natural products (e.g., **1**). Further studies are in progress in our research group.

Experimental

General

All reactions requiring anhydrous conditions were performed under a positive pressure of dry Ar using oven-dried glassware (>12 h, 120 °C) which was cooled under Ar. All organic layers obtained from extractions were dried over Na_2SO_4 or $MgSO_4$. Solvents for anhydrous reactions were dried according to Perrin *et al.*[213] Specifically, benzene, tetrahydrofuran (THF) and diethyl ether were distilled from sodium using benzophenone as the indicator. Acetonitrile, pyridine, triethylamine, and diisopropylethylamine were distilled from CaH_2. Anhydrous ethyl alcohol and methyl alcohol were prepared by distilling from Mg with a catalytic amount of iodine. Solvents used for chromatography were distilled. Water used was Milli-Q (Millipore) quality, which when necessary was degassed by heating *in vacuo* and cooling under Ar.

All reagents employed were ACS grade or finer. Air sensitive reagents were handled under an atmosphere of dry Ar. Diisopropylamine and hexamethydisilazane were distilled from CaH_2. Ethylenediamine was predried over CaO and KOH and then distilled from sodium. *N*,*N*-Dimethylformamide (DMF) was refluxed over CaH_2 for 12 h and then distilled under reduced pressure. *p*-Toluenesulfonic acid is used in its monohydrate form unless specified. All commercial organometallic reagents were obtained from Aldrich Chemical Co. *n*-Butyllithium solution was stored under Ar at room temperature and periodically titrated against menthol/phenanthroline.[214] Amino acids and amino acid derivatives used as starting materials were obtained from Sigma Chemical Co.

When possible, the progress of reactions was monitored by thin-layer chromatography (TLC) using one or more of the following for visualization: UV

absorption by fluorescence quenching; iodine staining; bromocresol green spray for acids; ninhydrin spray for amino acids; dodecamolybdophosphoric acid spray for general hydrocarbons. All spray reagents were prepared and used as described by Krebs *et al.*[215] For TLC of amino acids on ion exchange resin, a mixed solvent of *n*-BuOH/AcOH/H_2O was used, the ratio depending on the specific amino acid. For monitoring reactions in water or other non-volatile solvents, the solvent was removed from the TLC plate *in vacuo* or by heating before developing.

Reactions involving *N*-protected β-lactones, the deprotected β-lactone tosylate salts, and the *N*-acylated derivatives were monitored by TLC using bromocresol green spray (0.04% in EtOH, made blue by NaOH)[215] followed by heating of the plate for detection of the β-lactone as a yellow spot on a blue background.

Commercial thin-layer and preparative layer chromatography plates were: normal silica, Merck 60 F-254; reverse-phase, Merck RP-8F_{254} S and Macherey-Nagel and Co.; ion exchange resin, Polygram^R Ionex-25 SA-Na. Silica gel for column chromatography was Merck type 60, 70-230 mesh or its equivalent from General Intermediates of Canada. Flash chromatography was performed according to Still *et al.*[216] using Merck type 60, 230-240 mesh silica gel. All solvent mixtures are listed as volume ratios. The cation exchange resin used was Bio-Rad AG 50W-X8 (H^+ form, 50-100 mesh).

High performance liquid chromatography (HPLC) employed a Hewlett Packard 1082B instrument fitted with a Waters radial compression 8 x 100 mm μ-Bondapak 125 Å, C_{18} reverse phase column and a UV detector set at 258 nm for obafluorin (**19**) and 254 nm for compound **79**. For preparative purposes on this instrument, a sample volume of 50-100 μL at a concentration of 10 mg/mL was injected each time. Acetonitrile used was of HPLC grade. Both acetonitrile and water contained 0.1% trifluoroacetic acid and the solvents used were previously degassed and filtered through 0.45 μm filters.

All literature compounds had IR, ^1H NMR, and MS consistent with the assigned structures. Melting points were uncorrected and determined on a Thomas Hoover or Buchi oil immersion apparatus using open capillary tubes. Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (10.00 cm, 0.9 mL) at ambient temperature. All specific rotations reported were measured at the sodium D line. Infrared spectra (IR) were recorded on a Nicolet 7199 FT-IR spectrometer. The stability study of the β-lactone tosylate salt **63** in aqueous solution was followed by FT-IR using 0.1 mm IR-Trans cells (Kodak, polycrystalline ZnS). Mass spectra (MS) were recorded on a Kratos AEI MS-50 (high resolution, electron impact ionization), MS-12 (chemical (NH_3) ionization, CI-MS), or MS-9 (fast atom bombardment with argon, POSFAB). The term 'Cleland's reagent' (as a matrix in FAB MS) refers to a 5:1 mixture of dithiothreitol and dithioerythritol. Microanalyses were obtained using a Perkin Elmer 240 CHN analyzer.

Nuclear magnetic resonance (NMR) spectra were measured on Bruker WP-80 (continuous wave), WH-200, AM-300, WM-360, WH-400 or WH-500 instruments in the specified solvent with tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) in D_2O as internal standard for ^1H NMR spectra. For ^{13}C NMR spectra, which were obtained on the WH-200, AM-300 or WH-400, the deuterated solvent peak was used as the reference.

Compounds labelled with a " * " were synthesized by Dr. C. Lowe in our research group; compounds labelled with a " † " were prepared by Dr. F. M. Martin, a former post-doctoral fellow in our group.

SQ 26,517, (3*S*,4*R*)-3-(Acetylamino)-4-methyl-2-oxetanone (1).

A mixture of **33** (54.0 mg, 0.20 mmol) in CH_2Cl_2 (5 mL) at -10 °C was treated with pyridine (0.040 mL, 0.50 mmol) and acetyl chloride (19.0 mg, 0.24 mmol). The mixture was kept at -10 °C for 1 h, allowed to warm to 0 °C over 2 h, and then kept at 20 °C for 7 h. The solvent was removed *in vacuo* to give an oily residue which was then partitioned between EtOAc and aqueous $KHSO_4$. The organic layer was dried over Na_2SO_4 and concentrated to a liquid that crystallized upon standing to **1** (24 mg, 84%): mp 94-96 °C (lit.[6] mp 105.5-107.0 °C); IR ($CHCl_3$ cast), 3280 (br), 1839, 1817, 1743, 1664, 1541 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.51 (br, 1 H, N*H*), 5.63 (dd, 1 H, *J* = 8, 6 Hz, C*H*NH), 4.90 (dq, 1 H, *J* = 6, 8 Hz, CH_3CH), 2.09 (s, 3 H, CH_3CO), 1.44 (d, 3 H, *J* = 6 Hz, CH_3CH); MS (FAB) 144 (MH^+).

(+)-Obafluorin (2).

To a suspension of the tosylate salt **62** (35.0 mg, 0.089 mmol) in dry CH_2Cl_2 (1.0 mL) cooled to -12 °C under an argon atmosphere was added the acid chloride **63** (29.0 mg, 0.13 mmol), followed by pyridine (14 μL, 0.17 mmol). The reaction mixture was stirred at -10 °C for 20 min, then warmed to 20 °C over 2 h and stirred at 20 °C overnight. The mixture was partitioned between water (25 mL) and EtOAc (25 mL). The organic phase was washed with water (25 mL), dried ($MgSO_4$), and evaporated *in vacuo*. HPLC purification (Waters radial compression 8 x 100 mm μ-Bondapak 125 Å, C_{18} reverse phase, gradient elution, 35% to 70% acetonitrile/water) and lyophilization yielded obafluorin (**2**) (19 mg, 57%) as a white powder. However, upon standing in aqueous acetonitrile over several hours some hydrolysis of the β-lactone ring occurred to give **64**. The sample was repurified by rapid HPLC (C_{18} reverse phase, isocratic elution, 55% acetonitrile/water). Obafluorin showed good biological activity against *Staphylococcus aureus* strains ATCC 25923 and ATCC 13565. With the exception of the extent of optical rotation, the properties of

obafluorin (**1**) were consistent with those reported in the literature data:[8] $[\alpha]_D$ +43 °, (c = 0.03, MeCN) (lit.[8] $[\alpha]_D$ +116 °, (c = 0.1, MeCN)); IR (CH_3CN cast) 1835, 1648, 1519, 1348 cm^{-1}; ^1H NMR (500 MHz, CD_3CN) δ 8.10 (d, 2 H, *J* = 9 Hz, Ar*H*), 7.44 (d, 2 H, *J* = 9 Hz, Ar*H*), 7.18 (d, 1 H, *J* = 8 Hz, Ar*H*). 7.04 (d, 1 H, *J* = 7 Hz, Ar*H*), 6.84 (dd, 2 H, *J* = 8, 7 Hz, Ar*H*), 6.77 (br s, 1 H, N*H*), 5.74 (dd, 1 H, *J* = 8, 6 Hz, -C*H*-NH-), 5.04 (m, 1 H, $ArCH_2$-C*H*-), 3.37, 3.21 (ABX system, 2 H, J_{AB} = 14 Hz, J_{AX} = 9 Hz, J_{BX} = 5 Hz, Ar-CH_2-); exact mass 340.0692 ($M^+ - H_2O$) (340.0695 calcd for $C_{17}H_{12}N_2O_6$), 314.0889 ($M^+ - CO_2$) (314.0902 calcd for $C_{16}H_{14}N_2O_5$).

(2*S*,3*R*)-2-Amino-3-hydroxy-4-(*p*-nitrophenyl)butanoic acid (6).

Aqueous hydrochloric acid (6 N, 110 mL) was added to **55** (978 mg, 2.3 mmol), and the mixture was heated to reflux overnight to give a yellow solution. The mixture was cooled to room temperature, diluted with water (50 mL), and washed with CH_2Cl_2 (3 x 100 mL). The aqueous phase was filtered through a small plug of glass wool and evaporated *in vacuo*. The residue was purified by ion exchange chromatography on AG 50W-X8 (H^+) resin by elution with water followed with 1 N NH_4OH solution. The fractions containing the amino acid (ninhydrin positive) were first concentrated *in vacuo* and then lyophilized to yield **6** (467 mg, 85%) as a powder: mp 233 °C (dec.); $[\alpha]_D$ +50 °, (c = 0.18, H_2O); IR (KBr disk) 3420 (br), 3220 (br), 2520 (br), 1985 (br), 1620, 1604, 1595, 1516, 1405, 1350 cm^{-1}; ^1H NMR (D_2O, 500 MHz) δ 8.06 (d, 2 H, *J* = 8 Hz, Ar*H*), 7.22 (d, 2 H, *J* = 8 Hz, Ar*H*), 4.22 (m, 1 H, 3-H), 3.61 (d, 1 H, *J* = 5.5 Hz, 2-H), 3.08 (dd, 1 H, *J* = 14, 3.5 Hz, 4-H), 2.84 (dd, 1 H, *J* = 14, 10 Hz, 4-H); ^{13}C NMR (D_2O/DCl, 75.5 MHz) δ 37.2 (t), 55.2 (d), 67.3 (d), 122.0 (d), 128.4 (d), 143.0 (s), 144.7 (s), 167.4 (s); MS (CI, NH_3) 241 (MH^+, 90). Anal. Calcd for $C_{10}H_{12}N_2O_5$: C, 50.00; H, 5.04; N, 11.66. Found: C, 50.00; H, 5.04; N, 11.57.

(2*S*)-1-Benzoyl-2-(*tert*-butyl)-3-methyl-4-imidazolidinone (14).

The literature procedure[159] was modified. A suspension of **52** (9.74 g, 31.6 mmol) in CH_2Cl_2 (70 mL) was shaken with 2 N NaOH (35 mL). After separation of the two phases, the amine in the CH_2Cl_2 layer was benzoylated by simultaneous additions of benzoyl chloride (4.50 g, 31.6 mmol) and 1 N NaOH (40 mL) with ice-cooling. The reaction mixture was then stirred at room temperature for 12 h. The two layers were separated, and the CH_2Cl_2 phase was washed with water (2 x 40 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give a white solid. The crude product was further purified by recrystallization from ethanol to afford **14** (7.09 g, 86%) as fine white crystals: mp 137.5-139 °C (lit.[159] mp 143-144 °C) ; $[\alpha]_D$ +125 ° (c = 1, CH_2Cl_2) (lit.[159] $[\alpha]_D$ +126 °); IR ($CHCl_3$ cast) 1708, 1655, 1650, 1399, 1376, 1303, 1258 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.58 (m, 2 H, Ar*H*), 7.48 (m, 3 H, Ar*H*), 5.60 (d, 1 H, *J* = 0.9 Hz, NC*H*N), 4.13 (d, 1 H, *J* = 15.6 Hz, NC*H*HCO), 3.84 (d, 1 H, *J* = 15.6 Hz, NCH*H*CO), 3.05 (s, 3 H, NCH_3), 1.09 (s, 9 H, C(CH_3)$_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.5, 169.2, 134.5, 131.4, 128.5, 128.0, 80.8, 52.9, 39.7, 31.5, 26.0; exact mass 203.0820 (M^+ - C_4H_9) (203.0820 calcd for $C_{11}H_{11}N_2O_2$). Anal. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76. Found C, 69.12; H, 7.79; N, 10.54.

***N*-[(*o*-Nitrophenyl)sulfenyl]-L-threonine (21).**

The procedure of Gordon *et al.*[35] was modified. L-Threonine (2.98 g, 25.0 mmol) was added to dioxane (31 mL) and 2 N NaOH (12.5 mL). To the vigorously stirred solution was added *o*-nitrophenylsulfenyl chloride (5.23 g, 27.5 mmol) in ten equal portions over 15 min, while 2 N NaOH (15 mL) was added dropwise. After an additional 5 min, the reaction mixture was diluted with water (100 mL) and extracted with EtOAc (3 x 50 mL). The aqueous solution was acidified to pH 2.5 with 10% $KHSO_4$ and was immediately extracted with EtOAc (3 x 50 mL). The combined

organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a yellow solid. Recrystallization from acetone/hexane afforded **21** (5.37 g, 79%) as yellow crystals: mp 141-144 °C (lit.[35] mp 145-148 °C); IR (KBr) 3300-2900 (br), 1741, 1330, 1285, 737 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 8.25 (m, 2 H, Ar*H*), 7.68 (m, 1 H, Ar*H*), 7.30 (m, 1 H, Ar*H*), 4.20 (m, 1 H, MeC*H*OH), 3.38 (d, 1 H, *J* = 4 Hz, C*H*NH), 1.40 (d, 3 H, *J* = 6 Hz, C*H*$_3$); exact mass 272.0468 (272.0469 calcd. for $C_{10}H_{12}N_2O_5S$). Anal. Calcd for $C_{10}H_{12}N_2O_5S$: C, 44.11; H, 4.44; N, 10.29; S, 11.77. Found: C, 44.12; H, 4.45; N, 9.99; S, 11.96.

(3*S*,4*R*)-3-[[(*o*-Nitrophenyl)sulfenyl]amino]-4-methyl-2-oxetanone (23).

The procedure by Vederas and Pansare[36] was adapted. A solution of 4-bromobenzenesulfonyl chloride (2.00 g, 8.0 mmol) in dry pyridine (14 mL) at 0 °C was added dropwise over 10 min to a solution of **21** (1.00 g, 3.9 mmol) in pyridine (14 mL) at -43 °C. After 1 h at -43 °C the mixture was warmed to 0 °C for 3 h. Ice water (50 mL) was added and the solution was acidified to pH 2. The mixture was immediately extracted with EtOAc (5 x 50 mL), and the combined extracts were dried (Na_2SO_4) and concentrated. Purification by flash chromatography (hexane/EtOAc, 6/4) gave **23** (550 mg, 56%) as a yellow solid: mp 123-127 °C (lit.[35] mp 134-135 °C); IR ($CHCl_3$ cast) 1816, 1512, 1337, 735 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.34 (m, 1 H, Ar*H*), 8.10 (m, 1 H, Ar*H*), 7.80 (m, 1 H, Ar*H*), 7.38 (m, 1 H, Ar*H*), 4.92 (quint., 1 H, *J* = 6 Hz, CH_3CH), 4.75 (dd, 1 H, *J* = 8, 6 Hz, C*H*NH), 3.52 (d, 1 H, *J* = 8 Hz, N*H*), 1.6 (d, 3 H, *J* = 6 Hz, C*H*$_3$); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 169.5 (s), 134.5 (d), 127.0 (d), 126.3 (d), 125.9 (d), 125.4 (d), 123.9 (d), 75.7 (d), 70.8 (d), 15.2 (q); exact mass 254.0358 (254.0361 calcd for $C_{10}H_{10}N_2O_4S$). Anal. Calcd for $C_{10}H_{10}N_2O_4S$: C, 47.24; H, 3.96; N, 11.02; S, 12.61. Found: C, 46.97; H, 3.82; N, 10.77; S, 12.56.

_N_-[(_o_-Nitrophenyl)sulfenyl]-L-_allo_-threonine (30).

A procedure similar to that used to prepare **21** was followed. Thus, L-*allo*-threonine (238.0 mg, 2.0 mmol) was added to dioxane (3 mL) and 2 N NaOH (1.2 mL). To the vigorously stirred mixture was added *o*-nitrophenylsulfenyl chloride (417 mg, 2.0 mmol) in small portions, while 1 N NaOH (3 mL) was added dropwise. After a further 10 min, the reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAc (2 x 10 mL). The remaining aqueous solution was then acidified with 10% $KHSO_4$ to pH 2.5 and immediately extracted with EtOAc (3 x 10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated to give **30** (416 mg, 76%) as a bright yellow solid: mp 139-141 °C; IR (KBr) 3300, 1714, 1333, 739 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 8.16 (m, 2 H, Ar*H*), 7.72 (m, 1 H, Ar*H*), 7.31 (m, 1 H, Ar*H*), 4.18 (m, 1 H, CH_3CHOH), 3.42 (d, 1 H, J = 4 Hz, C*H*NH), 1.30 (d, 3 H, J = 7.6 Hz, CH_3); exact mass 272.0464 (272.0467 calcd for $C_{10}H_{12}N_2O_5S$). Anal. Calcd for $C_{10}H_{12}N_2O_5S$; C, 44.11; H, 4.44; N, 10.29; S, 11.77. Found: C, 43.85; H, 4.35; N, 9.99; S, 11.46.

Phenylsulfenyl Chloride (31).

The literature method[150] was followed. Freshly distilled sulfuryl chloride (10.2 g, 75.0 mmol) was slowly added at room temperature to a solution of diphenyl disulfide (16.2 g, 75.0 mmol) and pyridine (1.5 mL) in CH_2Cl_2 (50 mL). After completion of the addition, the solution was stirred for an additional 1.5 h. The solvent was removed *in vacuo* and the residue was distilled to give **31** (17.5 g, 82%) as a deep red liquid: bp 44-46 °C (3.5 mm Hg) [lit.[150] bp 49 °C (4 mm Hg)]; IR ($CHCl_3$ cast) 3010, 1575, 1470, 1435, 1160, 1140, 739, 681 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 7.65 (m, 2 H, Ar*H*), 7.35 (m, 3 H, Ar*H*); exact mass 143.9803 (143.9800 calcd for C_6H_5ClS).

(3*S*,4*S*)-3-[[(*o*-Nitrophenyl)sulfenyl]amino]-4-methyl-2-oxetanone (32).

A solution of **30** (289 mg, 1.06 mmol) in anhydrous pyridine (3.5 mL) was cooled to 0 °C. To this solution was added dropwise a solution of 4-bromobenzenesulfonyl chloride (827 mg, 3.18 mmol) in pyridine (3.5 mL) at 0 °C. The reaction was stirred at 0 °C for 3.5 h, and the solvent was removed under high vacuum. The residue was purified by flash chromatography (5-20% EtOAc/hexane) to afford **32** (109 mg, 41%) as a yellow solid: mp 107-110 °C; IR (KBr) 3300 (br), 1800, 1512, 1338, 1138, 736 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.30 (m, 1 H, Ar*H*), 8.07 (m, 1 H, Ar*H*), 7.74 (1 H, m, Ar*H*), 7.34 (m, 1 H, Ar*H*), 4.70 (m, 1 H, C*H*CH_3), 4.41 (dd, 1 H, *J* = 7, 4 Hz, C*H*NH), 3.72 (d, 1 H, *J* = 7 Hz, N*H*), 1.61 (d, 3 H, *J* = 7 Hz, CH_3); exact mass 254.0357 (254.0361 calcd for $C_{10}H_{10}N_2O_4S$). Anal. Calcd for $C_{10}H_{10}N_2O_4S$: C, 47.24; H, 3.96; N, 11.02; S, 12.61. Found: C, 47.43; H, 3.89; N, 10.88; S, 12.60.

Deprotection of 23 to (3*S*,4*R*)-3-Amino-4-methyl-2-oxetanone *p*-Toluenesulfonate Salt (33), 4-Methylphenyl 2-Nitrophenyl Disulfide (35)†, and 2-Nitrophenyl Disulfide (36)†.

To a stirred suspension of **23** (100 mg, 0.40 mmol) in CH_2Cl_2 (1 mL) (concentration important) under an argon atmosphere was added anhydrous *p*-toluenesulfonic acid (74 mg, 0.43 mmol) followed by *p*-thiocresol (100 mg, 0.80 mmol). The mixture was kept at 20 °C for 5 h, the solvent was evaporated, and the resulting yellow solid was triturated with diethyl ether until it was colorless. Recrystallization from EtOAc/hexane yielded **33** (81 mg, 75%): mp ca. 120 °C (dec.); IR (KBr) 3100 (br), 1841, 1204 cm^{-1}; ^1H NMR (360 MHz, DMF-d_7) δ 7.59 (d, 2 H, *J* = 7 Hz, Ar*H*), 7.08 (d, 2 H, *J* = 7 Hz, Ar*H*), 5.45 (d, 1 H, *J* = 7 Hz, C*H*NH_3), 5.10 (quint., 1 H, *J* = 7 Hz, CH_3C*H*), 2.25 (s, 3 H, ArCH_3), 1.65 (d, 3 H, *J* = 7

Hz, C*H*$_3$); MS (FAB) (glycerol) 274 (MH^+). Anal. Calcd for $C_{11}H_{15}NO_5S$: C, 48.35; H, 5.49; N, 5.12; S, 11.72. Found: C, 48.17; H, 5.36; N, 4.90; S, 11.34.

Thiophenol could be used in the above procedure in place of *p*-thiocresol to afford variable yields (65-92 %) of **33** with identical properties.

Evaporation of the combined ether layers from the trituration procedure and purification of the residue by repeated preparative TLC (hexane/EtOAc; 9/1) gave unsymmetrical disulfide **35** (13.9 mg, 13% based on **23**) and symmetrical disulfide **36** (2.7 mg, 2%). For **35**: IR ($CHCl_3$ cast) 1590, 1565, 1489, 1337, 1305, 799 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.20 (m, 2 H, Ar*H*), 7.60 (m, 1 H, Ar*H*), 7.35 (m, 3 H, Ar*H*), 7.08 (d, 2 H, *J* = 8 Hz, Ar*H*), 2.25 (s, 3 H, C*H*$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 137.9 (d), 137.3 (s), 134.1 (d), 130.0 (d), 129.7 (d), 128.5 (s), 128.3 (s), 127.0 (d), 126.3 (d), 126.0 (s), 21.0 (q); exact mass 277.0230 (277.0230 calcd for $C_{13}H_{11}NO_2S_2$).

For **36**: IR ($CHCl_3$ cast) 1587, 1566, 1505, 1333, 777 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.33 (dd, 2 H, *J* = 8.2, 1.5 Hz), 7.79 (dd, 2 H, *J* = 8.2, 1.4 Hz), 7.55 (dd, 1 H, *J* = 8.2, 1.4 Hz), 7.50 (dd, 1 H, *J* = 8.2, 1.5 Hz), 7.36 (dd, 1 H, *J* = 8.3, 1.4 Hz), 7.33 (dd, 1 H, *J* = 8.3, 1.4 Hz); exact mass 307.9926 (307.9926 calcd for $C_{12}H_8N_2O_4S_2$).

(3*S*,4*S*)-3-Amino-4-methyl-2-oxetanone *p*-Toluenesulfonate Salt (34).

A similar procedure to convert **23** to **33** was employed. To a solution of **32** (50.0 mg, 0.20 mmol) in CH_2Cl_2 (3 mL) was added *p*-toluenesulfonic acid (37.5 mg, 0.20 mmol) and *p*-thiocresol (50.0 mg, 0.40 mmol). The resulting suspension was stirred at room temperature for 4 h. The solvent was removed *in vacuo* and the residue was triturated with diethyl ether until the washings were colorless to afford the product **34** as a cream-colored solid (41.1 mg, 76%): mp ca. 120 °C (dec); IR (KBr) 3000-2800, 1831, 1219, 1172 cm^{-1}; ^1H NMR (200 MHz, DMF-d_7) δ 7.65 (d, 2 H, *J* = 8

Hz, Ar*H*), 7.13 (d, 2 H, *J* = 8 Hz, Ar*H*), 5.10 (m, 2 H, C*H*NH_3, CH_3CH), 2.30 (s, 3 H, ArCH_3), 1.65 (d, 3 H, *J* = 8 Hz, CH_3); FAB MS (glycerol) 274 (MH^+).

o-Nitrothiophenol (37).

The literature procedure[153] was modified. To a suspension of 2-nitrophenyl disulfide (4.00 g, 13.0 mmol) in MeOH (75 mL) were added 2-mercaptoethanol (2.0 mL, 28.5 mmol) and triethylamine (0.10 mL, 0.72 mmol). The mixture was stirred under Ar overnight and then filtered. Methanol was removed *in vacuo* from the filtrate, and the residue was diluted with a precooled (0 °C) 1 N HCl solution (50 mL) to give a yellow precipitate. The solid was collected by filtration and purified by flash chromatography ($CHCl_3$ as eluent) to afford the thiol **37** (2.0 g, 50%) as a yellow solid: mp 55-56 °C (lit.[153] mp 56 °C); IR (KBr disk) 2531, 1592, 1568, 1517, 1503, 1455, 1333, 1309, 1115, 852, 784, 732 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.26 (d, *J* = 8.0 Hz, 1 H, Ar*H*), 7.45 (m, 2 H, Ar*H*), 7.30 (m, 1 H, Ar*H*), 4.03 (s, 1 H, S*H*); exact mass 155.0033 (155.0041 calcd for $C_6H_5NO_2S$). Anal. Calcd for $C_6H_5NO_2S$: C, 46.44; H, 3.25; N, 9.03; S, 20.66. Found: C, 46.37; H, 3.23; N, 8.86; S, 20.78.

(3*S*,4*R*)-3-(Benzoylamino)-4-methyl-2-oxetanone (38).

A suspension of **33** (110 mg, 0.39 mmol) in CH_2Cl_2 (3 mL) at 0 °C under argon was treated with benzoyl chloride (0.070 mL, 0.60 mmol) followed by pyridine (0.060 mL, 0.74 mmol). The solution was stirred at 0 °C for 1 h and then warmed to 20 °C overnight. EtOAc (30 mL) was added and the solution was washed with water (3 x 10 mL). The organic extract was dried (Na_2SO_4) and evaporated *in vacuo*. The resulting residue was triturated with diethyl ether to yield solid **38** (68 mg, 85%): mp 157-159 °C; IR (KBr) 3261, 1839, 1808, 1641, 1596, 1289, 720, 680 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.82 (m, 2 H, Ar*H*), 7.58 (m, 3 H, Ar*H*); 5.44 (dd, 1 H,

J = 6, 8 Hz, C*H*NH), 5.00 (quint, 1 H, *J* = 6 Hz, CH_3CH), 1.45 (d, 3 H, *J* = 6 Hz, CH_3); MS (CI, NH_3) 223 (MNH_4^+, 8.7), 206 (MH^+, 100).

(3*S*,4*R*)-3-[[*N*-(*tert*-Butoxycarbonyl)-D-phenylalaninyl]amino]-4-methyl-2-oxetanone (39).

A solution of *N*-(*tert*-butoxycarbonyl)-D-phenylalanine (26.5 mg, 0.10 mmol) in CH_2Cl_2 (3.0 mL) at -5 °C was treated with triethylamine (10.0 mg, 0.10 mmol) and ethyl chloroformate (11.0 mg, 0.10 mmol). The solution was stirred 20 min and **33** (27.3 mg, 0.10 mmol) and pyridine (0.020 mL, 0.20 mmol) were added. After 30 min at -5 °C, the solution was allowed to warm to 20 °C overnight. The solvent was removed and the residue was triturated with EtOAc (3 x 5 mL). The combined organic extracts were washed with water (10 mL), dried (Na_2SO_4), and concentrated *in vacuo* to afford a solid. This was triturated first with hexane and then with 10:1 hexane:ether (ca. 2 mL) to afford **39** (32.0 mg, 92%): mp 156-157 °C; IR ($CHCl_3$ cast), 3328, 2979, 1825, 1686, 1665 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.24 (m, 5 H, Ar*H*), 7.01 (d, 1 H, *J* = 8 Hz, N*H*), 5.57 (dd, 1 H, *J* = 8, 6 Hz, C*H*NH), 4.92 (d, 1 H, *J* = 8 Hz, N*H*), 4.80 (quint., 1 H, *J* = 6 Hz, CH_3CH), 4.38 (q, 1 H, *J* = 8 Hz, C*H*NHBoc), 3.06 (m, 2 H, $PhCH_2CH$), 1.40 (s, 9 H, $NHCOOC(CH_3)_3$), 1.22 (d, 3 H, *J* = 6 Hz, CH_3CH); FAB MS m/z 349 (MH^+). Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 62.13; H, 6.72; N, 8.06.

(3*S*,4*R*)-3-[[*N*-(*tert*-Butoxycarbonyl)-L-phenylalaninyl]amino]-4-methyl-2-oxetanone (40).

The procedure used to prepare **39** was employed to condense *N*-(*tert*-butoxycarbonyl)-L-phenylalanine with **33**. Thus, to a solution of *N*-*t*-Boc-L-phenylalanine (26.5 mg, 0.10 mmol) in CH_2Cl_2 (3 mL) at -5 °C was added triethylamine (10.0 mg, 0.10 mmol) and ethyl chloroformate (11.0 mg, 0.10 mmol).

The solution was stirred for 20 min before **33** (27.3 mg, 0.10 mmol) and pyridine (0.020 mL, 0.20 mmol) were added. After 30 min at -5 °C, the solution was allowed to warm up to room temperature overnight. The solvent was removed *in vacuo* and the residue was triturated with EtOAc (3 x 5 mL). The combined organic extracts were washed with water (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford **40** as a white solid (32.1 mg, 92%): mp 144-145 °C; IR ($CHCl_3$ cast) 3334, 1827, 1677 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.25 (m, 6 H, Ar*H* and N*H*), 5.52 (dd, 1 H, *J* = 8, 6 Hz, C*H*NH), 5.11 (d, 1 H, *J* = 8 Hz, N*H*), 4.85 (quint., 1 H, *J* = 6 Hz, CH_3CH), 4.39 (q, 1 H, *J* = 8 Hz, C*H*NHBoc), 3.07 (m, 2 H, $PhCH_2CH$), 1.40 (s, 9 H, $NHCOOC(CH_3)_3$), 1.33 (d, 3 H, *J* = 6 Hz, CH_3CH); FAB MS m/z 349 (MH^+). Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 61.73; H, 6.74; N, 7.92.

Hydrobromide Salt of (2*R*,3*S*)-2-Amino-3-bromobutanoic Acid (41).

Compound **41** could be obtained by three methods.

1.† A 30% solution of HBr in acetic acid (0.11 mL, 1.70 mmol) was added to **23** (86.0 mg, 0.34 mmol) and the mixture was stirred at 20 °C for 15 min. The acetic acid was evaporated *in vacuo* and EtOAc (25 mL) was added. This solution was extracted with water (3 x 20 mL) and concentrated *in vacuo* to yield solid **41** (42 mg, 68%): mp 179-183 °C (dec) (lit.[156] mp 198 °C); IR (KBr) 3067 (br), 1733, 1482, 1201 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.60 (dq, 1 H, *J* = 8, 4 Hz, CH_3CH), 4.31 (d, 1 H, *J* = 4 Hz, C*H*NH_3), 1.72 (d, 3 H, *J* = 8 Hz, CH_3); FAB (glycerol) 181.96, 183.97 [MH^+ - HBr, (^{79}Br) (^{81}Br)].

2. A 30% solution of HBr in acetic acid (0.13 mL, 1.90 mmol) was added to **33** (100 mg, 0.37 mmol) and the mixture was stirred at 20 °C for 15 min. The solvent was evaporated to yield a solid which after trituration with diethyl ether yielded **41** (90 mg, 92%) with identical properties.

3. A solution of **33** (10.0 mg, 0.037 mmol) in concentrated aqueous HBr (1 mL) was stirred at room temperature for 10 min. The solvent was removed *in vacuo*, and the residue was triturated with diethyl ether (6 x 5 mL) to give **41** in quantitative yield with identical properties.

Hydrobromide Salt of (2*R*,3*R*)-2-Amino-3-bromobutanoic Acid (42).†

The procedure described above for the preparation of **41** from **23** was used to convert **32** to **42** in 69% yield except that an 18 h reaction time was required: mp 165 °C (dec); IR (CH_3CN cast) 3000-2800 (br), 1737, 1488, 1211 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.75 (m, 1 H, CH_3CH), 4.20 (d, 1 H, J = 4 Hz, $CHNH_3$), 1.75 (d, 3 H, J = 7 Hz, CH_3); FAB MS 181.96, 183.96 [MH^+ - HBr, (^{79}Br)(^{81}Br)].

***p*-Tosylate Salt of (2*R*, 3*S*)-2-Amino-3-chlorobutanoic Acid (43) and *p*-Tosylate Salt of L-Threonine (44)**

A solution of **33** (54.4 mg, 0.20 mmol) in concentrated aqueous HCl (1 mL) was stirred for 20 min. The solvent was removed *in vacuo* to give a solid residue. Trituration with ether provided a solid mixture of **43** (78 %) and L-threonine tosylate (**44**, 22 %) as indicated by ^1H NMR (200 MHz). IR (KBr disk) 3442, 3431, 3700-2400 (br), 1736, 1605, 1580, 1511, 1241, 1222, 1212, 1161, 1145, 1122, 1034, 1010, 687, 566 cm^{-1}. For **43**: ^1H NMR (200 MHz, CD_3OD) δ 7.64 (d, 2 H, J = 8.0 Hz, Ar*H*), 7.15 (d, 2 H, J = 8.0 Hz, Ar*H*), 4.58 (m, 1 H, 3-H), 4.35 (d, 1H, J = 3.5 Hz, 2-H), 2.33 (s, 3 H, ArCH_3), 1.68 (d, 3 H, J = 6.0 Hz, CH_3CH); exact mass 92.0268 (M^+ - (CO_2H + TsOH)) (92.0267 calcd for C_3H_7NCl), FAB MS m/z 310 and 312 (MH^+, ^{35}Cl and ^{37}Cl). For **44**: ^1H NMR (200 MHz, CD_3OD) δ 7.64 (d, 2 H, J = 8.0 Hz, Ar*H*), 7.15 (d, 2 H, J = 8.0 Hz, Ar*H*), 4.24 (m, 1 H, 3-H), 3.78 (d, 1 H, J = 4 Hz, 2-H), 2.33 (s, 3 H, ArCH_3), 1.31 (d, 3 H, J = 6.0 Hz, CH_3CH); MS (FAB) 292 (MH^+).

(2*R*,3*S*)-3-Bromo-2-(benzoylamino)butanoic Acid (45).†

A solution of **38** (65.0 mg, 0.32 mmol) in freshly distilled THF (5.0 mL) was added dropwise at 20 °C to a suspension of anhydrous $MgBr_2 \cdot OEt_2$ (1.30 mmol) (prepared by addition of freshly distilled 1,2-dibromoethane (0.12 mL, 1.3 mmol) to Mg metal (32.0 mg, 1.30 mmol) in diethyl ether (5.0 mL)). After 10 min, the mixture was cooled to 4 °C and acidified with 1 M H_3PO_4 (6 mL). The phases were separated and the aqueous phase was extracted with ether (3 x 10 mL). The organic extracts were combined, dried (Na_2SO_4), and concentrated to yield a colorless oil **45** (86.0 mg, 94%). This material could be purified by preparative TLC (formic acid/methanol/$CHCl_3$, 1 : 9 : 90) but was unstable and decomposed rapidly at room temperature: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.80 (br s, 1 H, CO_2H), 7.85 (m, 2 H, Ar*H*), 7.52 (m, 3 H, Ar*H*), 7.00 (d, 1 H, *J* = 8 Hz, N*H*), 5.08 (dd, 1 H, *J* = 8, 4 Hz, C*H*NH), 4.55 (quint., 1 H, *J* = 4 Hz, CH_3CH), 1.95 (d, 3 H, *J* = 8 Hz, CH_3).

3-(*tert*-Butyl) (*R*)-4-Formyl-2,2-dimethyl-3-oxazolidine-carboxylate (46).

The literature procedure[60] was employed. To a stirred solution of oxazolidine ester **48** (2.00 g, 7.70 mmol) in dry toluene (15 mL) at -78 °C was added DIBAL in hexane (1.0 M, 13.1 mL, 13.1 mmol). The rate of addition was adjusted so as to keep the internal temperature below -65 °C. The reaction mixture was stirred at -78 ° C for 10 h. TLC in (4:1) hexane-EtOAc showed the clean formation of product **46** (R_f = 0.35) with only a trace of starting material (R_f = 0.40). The reaction was quenched by slow addition of MeOH (3 mL) (H_2 evolution!) at a rate such that the internal temperature was kept below -65 °C. The resulting white emulsion was slowly poured into ice-cold 1 N HCl (50 mL) with swirling over 5-10 min, and the aqueous mixture was extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with saturated NaCl solution (50 mL), dried over Na_2SO_4, and concentrated *in vacuo*

to give the crude **46** as a colorless oil. This material was vacuum distilled through a 10 cm Vigreux column to give pure oxazolidine aldehyde **46** (0.74 g, 42%) as a colorless liquid: bp 90-92 °C (2 mm Hg) (lit.[60] bp 83-88 °C (1.0-1.4 mm Hg)); IR ($CHCl_3$ cast) 3450, 2979, 2936, 1739, 1707, 1694, 1479, 1458, 1393, 1379, 1367, 1259, 1208, 1173, 1095, 1080, 850 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.59 (m, 1 H, C*H*O), 4.60-4.18 (m, 1 H, C*H*NCO), 4.08 (m, 2 H, OCH_2), 1.70-1.40 (m, 15 H, OC(CH_3)$_2$N and NCO$_2$C(CH_3)$_3$); exact mass 200.1291 (M^+ - CHO) (200.1286 calcd for $C_{10}H_{18}NO_3$), 144.0662 (M^+ - (CO + $C(CH_3)_3$), 100) (144.0661 calcd for $C_6H_{10}NO_3$). Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.63; H, 8.35; N, 6.11. Found: C, 57.46; H, 8.24; N, 5.95.

_N_-[(*tert*-Butoxy)carbonyl]-D-serine Methyl Ester (47).

The literature procedure[60] was employed. A solution of di-*tert*-butyl dicarbonate (39.2 g, 0.18 mol) in dioxane (140 mL) was added to a vigorously stirred solution of D-serine (15.9 g, 0.15 mol) in 1 N NaOH (310 mL) at 0 °C. The two-phase mixture was stirred at 5 °C for 30 min, then allowed to warm to room temperature over 4 h. The mixture was concentrated to half its original volume *in vacuo* at 35 °C, cooled to 0 °C, acidified to pH 2-3 by slow addition of 1 M $KHSO_4$, and then extracted with EtOAc (3 x 300 mL). The combined extracts were dried over $MgSO_4$, filtered, and concentrated to give *N*-Boc-D-serine (32.8 g, containing small amount of starting material as shown by ^1H NMR). The crude product was used in the next step without further purification.

To a cold solution of the crude *N*-Boc-D-serine (32.8 g, ca. 0.15 mol) in DMF (150 mL) was added solid K_2CO_3 (24.3 g, 0.18 mol). The reaction mixture was stirred for 10 min in an ice-water bath, and methyl iodide (20.0 mL, 46.3 g, 0.33 mol) was added to the white suspension. After 30 min at 0 °C, the mixture was warmed to

room temperature and stirred for an additional hour at which point TLC indicated the completion of the reaction. The reaction mixture was vacuum-filtered, and the filtrate was partitioned between EtOAc (300 mL) and water (300 mL). The organic phase was washed with saturated NaCl solution (2 x 300 mL), dried over $MgSO_4$, filtered, and concentrated to an amber oil. Purification by flash chromatography (10-20% EtOAc/hexane) afforded **47** (17.2 g, 52%) as an oil: IR ($CHCl_3$ cast) 3400, 2978, 1745, 1714, 1692, 1512, 1503, 1367, 1350, 1211, 1163, 1060 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.53 (br d, 1 H, *J* = 8 Hz, N*H*), 4.37 (m, 1 H, C*H*NH), 3.93 (m, 2 H, HOC*H*$_2$), 3.78 (s, 3 H, CO_2CH_3), 2.69 (br s, 1 H, *H*OCH$_2$), 1.46 (s, 9 H, NHCO$_2$C(C*H*$_3$)$_3$); exact mass 189.1000 (M^+ - CHO) (189.1001 calcd for $C_8H_{15}NO_4$), MS (CI) 220 (MH^+).

3-(*tert*-Butyl) 4-Methyl (*R*)-2,2-Dimethyl-3,4-oxazolidine-dicarboxylate (48).

The literature procedure[60] was employed. A solution of *N*-Boc-L-serine methyl ester (**47**) (5.90 g, 26.9 mmol), 2,2-dimethoxypropane (DMP, 6.7 mL, 5.70 g, 54.5 mmol) and *p*-toluenesulfonic acid monohydrate (0.112 g, 0.589 mmol) in benzene (94 mL) was heated at reflux for 30 min and then slowly distilled over 3 h to collect 80 mL of distillate. A TLC check of the cooled reaction mixture in (1 : 1) EtOAc-hexane showed both the formation of the desired product (R_f = 0.8) and the starting material (R_f = 0.5). More reagents (DMP, 2 mL, 15.7 mmol; benzene, 40 mL) were added to the reaction mixture and the distillation was continued until TLC showed completion of the reaction. The cooled amber solution was partitioned between saturated $NaHCO_3$ solution (12 mL) and Et_2O (73 mL). The organic layer was washed with saturated $NaHCO_3$ solution (25 mL) followed by saturated NaCl solution (15 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give an amber oil (7.34 g). This material was vacuum-distilled through a 10 cm Vigreux column to give pure **48** (4.96 g, 71%) as a

colorless oil: bp 92-95 °C (1.5-2 mm Hg) (lit.[48] bp 101-102 °C (2 mm Hg)); IR ($CHCl_3$ cast) 2979, 1759, 1710, 1392, 1381, 1367, 1270, 1253, 1205, 1175, 1094, 1068, 1055 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.55-4.35 (m, 1 H, C*H*NC), 4.20-4.00 (m, 2 H, OCH_2), 3.78 (s, 3 H, CO_2CH_3), 1.46-1.40 (m, 15 H, $OC(CH_3)_2N$, and $CO_2C(CH_3)_3$); exact mass 244.1184 (M^+ - CH_3) (244.1185 calcd for $C_{11}H_{18}NO_5$), 144.0661 (M^+ - (CH_3 + $CO_2C(CH_3)_3$), 100) (144.0661 calcd for $C_6H_{10}NO_3$). Anal. Calcd for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.32; H, 8.37; N, 5.46.

Glycine *N*-Methylamide (49).

The literature procedure[159] was used. To a solution of methylamine (85.2 g, 2.75 mol) in ethanol (500 mL) at 0 °C (prepared by passing the amine gas into absolute ethanol at 0 °C) was added a suspension of glycine ethyl ester hydrochloride (55.8 g, 0.40 mmol) in ethanol (1000 mL). The clear greenish solution was stirred at 0 °C for 5.5 h and then allowed to warm to room temperature for 48 h. The solution was concentrated to about 500 mL *in vacuo* to give a cloudy suspension, and after the mixture had been cooled to -20 °C, Et_2O (800 mL) was added in several small portions to induce more precipitation of monomethylamine hydrochloride. The removal of the solid by filtration and the concentration of the filtrate afforded pure **49** (34.5 g, 98%) as an oil: IR (neat) 3280 (br, s) 3082, 2949, 1662, 1545, 1411, 1271, 1159, 911, 882 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 3.84 (s, 2 H, H_2NCH_2CO), 3.35 (s, 3 H, $NHCH_3$); exact mass 88.0640 (M^+, 100) (88.0637 calcd for $C_3H_8N_2O$). Anal. Calcd for $C_3H_8N_2O$: C, 40.90; H, 9.15; N, 31.79. Found: C, 40.77; H, 8.93; N, 31.58.

***N*-Methyl-2-[(2,2-dimethylpropylidene)amino]ethanamide (50).**

The literature procedure[159] was used. A suspension of **49** (34.1 g, 0.34 mol) in pentane (600 mL) was treated with pivalaldehyde (43.4 g, 0.50 mol). The mixture was heated to reflux using a Dean-Stark apparatus to remove the water formed. After 7 h, the reaction solution was concentrated *in vacuo* to give **50** (54.2 g, 90%) as a clear liquid: IR ($CHCl_3$ cast) 3300, 2955, 2903, 1663, 1544, 1410 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.60 (m, 1 H, N=C*H*), 6.88 (br s, 1 H, -N*H*CH_3), 4.02 (s, 2 H, NCH_2CO), 2.89 (d, 3 H, J = 5.0 Hz, NHCH_3), 1.10 (s, 9 H, C(CH_3)$_3$); exact mass 99.0559 (M^+ - $C(CH_3)_3$) (99.0558 calcd for $C_4H_7N_2O$). Anal. Calcd for $C_8H_{16}N_2O$: C, 61.51; H, 10.32; N, 17.93. Found: C, 61.38; H, 10.21; N, 17.72.

(*R*,*S*)-2-(*tert*-Butyl)-3-methyl-4-imidazolidinone (51).

The literature procedure[159] was used. MeOH (115 mL) saturated with HCl was prepared by passing HCl gas into cold MeOH (0 °C). To this solution (70.8 g, 1.94 mol) was added with cooling (0 °C) a solution of **50** (19.2 g, 0.12 mol) in MeOH (90 mL). The reaction mixture was stirred at 0 °C for 2 h and then at room temperature overnight. The solvent was removed *in vacuo* to give the hydrochloride salt (23.7 g) as a solid. This material was suspended in CH_2Cl_2 (180 mL) and extracted with 3 N NaOH solution (3 x 100 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL) and the combined organic phases were washed with saturated NaCl solution (2 x 100 mL), dried over Na_2SO_4, and concentrated *in vacuo* to afford **51** (11.7 g, 66%) as an oil: IR ($CHCl_3$ cast) 3340, 2960, 1620, 1400, 1320, 1100, 1055, 1045 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.12 (s, 1 H, NC*H*NH), 3.48 (d, 2 H, J = 5 Hz, NHCH_2CO), 2.94 (s, 3 H, NCH_3), 2.10 (br s, 1 H, N*H*), 0.98 (s, 9 H, C(CH_3)$_3$); exact mass 141.1026 (M^+ - CH_3) (141.1028 calcd for $C_7H_{13}N_2O$), 99.0558 (M^+ - $C(CH_3)_3$, 100) (99.0558 calcd for $C_4H_7N_2O$). Anal. Calcd for $C_8H_{16}N_2O$: C, 61.51; H, 10.32; N, 17.93. Found: C, 61.33; H, 10.08; N, 17.68.

(2*R*)-2-(*tert*-Butyl)-3-methyl-4-imidazolidinon-1-yl (R)-Mandelate (52).

The literature procedure[159] was modified. A mixture of **51** (9.30 g, 59.6 mmol) and *R*-(-)-mandelic acid (9.30 g, 61.2 mmol) was dissolved in boiling acetone (50 mL). The solution was allowed to cool to room temperature over several hours and needle-shaped crystals were formed along the side of the flask. This facilitated the separation of the (*R*) amine (*R*) mandelate salt **52** (solid phase) from the (*S*) amine (*R*) mandelate salt **52a** (liquid phase). After 14 h at 4 °C, the crystals were collected by filtration and vacuum-dried (7.20 g, 78%). mp 104-106 °C; IR ($CHCl_3$ cast) 3220, 3040, 2958, 1707, 1645, 1041, 740, 402 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.62-7.40 (m, 5 H, Ar*H*), 5.60 (s, 1 H, NC*H*N), 4.13 (d, 1 H, *J* = 16 Hz, NC*H*HCO), 3.75 (d, 1 H, J = 16 Hz, NCH*H*CO), 3.05 (s, 3 H, NCH_3), 1.11 (s, 9 H, $C(CH_3)_3$); MS (FAB) 309 (MH^+); Anal. Calcd for $C_{16}H_{24}N_2O_4$: C, 62.32; H, 7.84; N, 9.08. Found: C, 62.13; H, 8.01; N, 8.96.

***p*-Nitrophenylacetaldehyde (53).**

The literature procedure[164] was modified. A solution of *p*-nitrostyrene (0.75 g, 5.0 mmol) in CH_2Cl_2 (10 mL) was added dropwise over 15 min to a well-stirred solution of $Pb(OAc)_4$ (2.22 g, 5.0 mmol) in trifluoroacetic acid (5 mL) at room temperature. After 3 h, the mixture was poured into water (25 mL) and was extracted with ether (3 x 10 mL). The combined organic extracts were washed with saturated $NaHCO_3$ solution (25 mL) and water (25 mL), and were dried over $MgSO_4$. Evaporation of the solvent *in vacuo* yielded **53** (0.66 g, 83%) as a solid: mp 82-84 °C (lit.[164] mp 83-85 °C); IR ($CHCl_3$ cast) 1709, 1516, 1495, 1387, 1343, 1316, 1106, 858, 719 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.83 (t, 1 H, *J* = 2.5 Hz, C*H*O), 8.24 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 7.40 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 3.88 (d, 2 H, *J* = 2.5 Hz, $ArCH_2CHO$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 197.1, 147.4, 141.9, 130.6, 124.0,

50.0; exact mass 165.0426 (165.0426 calcd for $C_8H_7NO_3$). Anal. Calcd for $C_8H_7NO_3$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.15; H, 4.16; N, 8.49.

_p_-Chlorophenylacetaldehyde (54).

The procedure similar to that used to prepare **53** was followed. A solution of *p*-chlorostyrene (1.39 g, 10.0 mmol) in CH_2Cl_2 (20 mL) was added dropwise over 30 min to a well-stirred solution of $Pb(OAc)_4$ (4.43 g, 10.0 mmol) in trifluoroacetic acid (10 mL) at room temperature. After 3 h, the mixture was poured into water (100 mL) and was extracted with ether (3 x 50 mL). The combined extracts were washed first with saturated $NaHCO_3$ solution until carbon dioxide evolution ceased and then with water (50 mL). The pale yellow solution was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the product **54** (1.49 g, 96%) as an oil which solidified at -20 °C: IR ($CHCl_3$ cast) 2926, 1784, 1733, 1724, 1493, 1224, 1169, 1153, 1092, 1015, 822, 805 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.76 (t, 1 H, *J* = 2.2 Hz, C*H*O), 7.35 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 7.15 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 3.67 (d, 2 H, *J* = 2.2 Hz, ArCH_2CHO); exact mass 154.0192 (154.0185 calcd for $C_8H_7{}^{35}ClO$), 156.0160 (156.0156 calcd for $C_8H_7{}^{37}ClO$).

(2*R*,5*S*,1'*R*)-5-[1'-Benzoyloxy-2'-(*p*-nitrophenyl)ethyl]-2-(*tert*-butyl)-3-methylimidazolidin-4-one (55).

The general aldol condensation procedure of Seebach *et al.*[159] using imidazolidinone **14** was adapted. To hexamethyldisilazane (1.47 mL, 6.97 mmol) in THF (4.0 mL) at -78 °C was added *n*-butyllithium (1.95 M in hexanes, 3.56 mL, 6.95 mmol). The solution was stirred at -78 °C for 15 min, then at 20 °C for 10 min. The solvent was removed under high vacuum over ca. 30 min. The resulting white powder was redissolved in THF (40 mL) and cooled to -78 °C; to this solution was added a solution of (*S*)-1-benzoyl-2-(*tert*-butyl)-3-methyl-4-imidazolidinone (**14**) (1.64 g, 6.32

mmol) in THF (21 mL) in a rapid dropwise manner, and the resulting orange/red solution was stirred at -78 °C for 30 min. A solution of chlorotris[diethylamino]titanium (2.06 g, 7.58 mmol) in sodium-dried hexane (6.3 mL) was added dropwise over ca. 10 min and then stirred at -78 °C for 1 h. The brown reaction mixture was cooled to -100 °C, and a solution of (*p*-nitrophenyl)-acetaldehyde (**53**) (1.25 g, 7.57 mmol) in THF (21 mL) was added dropwise over 20 min. The reaction mixture was warmed to -78 °C over ca. 10 min and stirred at this temperature for 3.5 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution (80 mL) with vigorous stirring, and the mixture was allowed to warm to room temperature over ca. 30 min. The mixture was diluted with water (80 mL) and extracted with diethyl ether (3 x 150 mL). The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was purified by flash chromatography (40-60% EtOAc/hexane) to give **55** (1.64 g, 61%) as a foam: $[\alpha]_D$ +120.5 °, (c = 1.0, CH_2Cl_2); IR ($CHCl_3$ cast) 3375 (br), 2960, 1721, 1694, 1520, 1346, 1269, 1109, 712 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.14 (d, 2 H, *J* = 12 Hz, NO_2-Ar*H*), 7.95 (d, 2 H, *J* = 10 Hz, Ar*H*), 7.58 (t, 1 H, *J* = 10 Hz, Ar*H*), 7.50 (d, 2 H, *J* = 12 Hz, NO_2-Ar*H*), 7.45 (t, 2 H, *J* = 10 Hz, Ar*H*), 5.55 (dt, 1 H, *J* = 5, 7 Hz, 1'-H), 4.22 (d, 1 H, *J* = 2 Hz, 2-H), 3.72 (dd, 1 H, *J* = 5, 2 Hz, 5-H), 3.33 (d, 2 H, *J* = 7 Hz, ArCH_2), 2.94 (s, 3 H, NCH_3), 0.98 (s, 9 H, $(CH_3)_3$C-); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 25.6 (q), 31.3 (q), 36.9 (s), 37.5 (t), 59.9 (d), 74.5 (d), 83.7 (d), 123.7 (d), 128.5 (d), 129.6 (d), 129.7 (s), 130.6 (d), 133.4 (d), 144.7 (s), 147.0 (s), 165.5 (s), 172.7 (s); MS (CI, NH_3) 426 (MH^+, 100). Anal. Calcd for $C_{23}H_{27}N_3O_5$: C, 64.93; H, 6.40; N, 9.88. Found: C, 64.69; H, 6.38; N, 9.52.

(2*R*,3*S*)-2-Amino-3-hydroxy-4-(*p*-nitrophenyl)butanoic acid (57).*

This was prepared in a similar manner to **6** from (*R*)-1-benzoyl-2-(*tert*-butyl)-3-methyl-4-imidazolidinone (**14a**) and (*p*-nitrophenyl)acetaldehyde (**53**), followed by acidic hydrolysis (6N HCl) of the adduct **56** and ion exchange chromatography to yield **57** as an off-white powder: $[\alpha]_D$ - 68 °, (c = 0.18, H_2O); remaining data as for **6**.

The spectral data (^{1}H NMR, IR and MS) for adduct **56** are identical to those of **55**.

Methyl (2*S*,3*R*)-3-Hydroxy-2-[[(1*S*,4*R*)-4,7,7-trimethyl-3-oxo-2-oxa-bicyclo[2.2.1]heptane-1-carbonyl]amino]-4-(*p*-nitrophenyl)-butanoate (58).*

The amino acid **6** (10 mg, 0.042 mmol) was dissolved in pH 10 sodium hydrogen carbonate/sodium carbonate buffer solution (1 M, 1.0 mL). A solution of (*S*)-camphanic acid chloride (18 mg, 0.083 mmol) in toluene (0.3 mL) was added at room temperature. The mixture was capped and stirred vigorously for 2.5 h. The reaction mixture was acidified to pH 1 with aqueous hydrochloric acid (6 N), and extracted with CH_2Cl_2 (4 x 3 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated *in vacuo* to yield a pale yellow solid. This residue was dissolved in ether (2 mL) and esterified by adding ethereal diazomethane until a yellow coloration persisted. The excess diazomethane was removed by bubbling argon into the solution for 10 minutes, then the solvent was evaporated *in vacuo* to afford a pale yellow oil. Methyl camphanoate was removed by sublimation under high vacuum (75 °C, 6 h, 0.3 mm Hg) to leave compound **58** (16.2 mg, 90%): IR (CH_2Cl_2 cast) 3425 (br), 2960, 2929, 1793, 1751, 1680, 1521, 1347 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, 2 H, *J* = 11 Hz, Ar*H*), 7.41 (d, 2 H, *J* = 11 Hz, Ar*H*), 7.24 (d, 1 H, *J* = 12 Hz, N*H*), 4.75 (dd, 1 H, *J* = 12, 2 Hz, 2-H), 4.44 (m, 1 H, 3-H), 3.78 (s,

3 H, CO_2CH_3), 2.87 (ABX system, 2 H, J_{AB} = 17 Hz, J_{AX} = 11 Hz, J_{BX} = 6 Hz, 4-H), 2.56-2.49 (m, 1 H, 6'-H_{exo}), 2.05-1.96 (m, 2 H, 6'-H_{endo}, 5'-H_{exo}), 1.77-1.71 (m, 1 H, 5'$_{endo}$), 1.16 (s, 3 H, 10'-CH_3), 1.15 (s, 3 H, 9'-CH_3), 1.04 (s, 3 H, 8'-CH_3); MS (EI) 435 (MH^+, 0.5); exact mass 435.1772 (MH^+) (435.1767 calcd for $C_{21}H_{27}N_2O_8$).

Methyl (2*R*,3*S*)-3-Hydroxy-2-[[(1*S*,4*R*)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carbonyl]amino]-4-(*p*-nitrophenyl)-butanoate (59).*

This was prepared from the amino acid **57** (10 mg, 0.042 mmol) in a manner analogous to that described above, to yield the title compound **59** (17.3 mg, 96%); IR (CH_2Cl_2 cast) 3427 (br), 2957, 2928, 1793, 1752, 1681, 1521, 1347 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.19 (d, 2 H, *J* = 11 Hz, Ar*H*), 7.42 (d, 2 H, *J* = 11 Hz, Ar*H*), 7.23 (d, 1 H, *J* = 12 Hz, N*H*), 4.75 (dd, 1 H, *J* = 12, 2 Hz, 2-H), 4.52 (m, 1 H, 3-H), 3.76 (s, 3 H, CO_2CH_3), 2.89 (d, 2 H, *J* = 9 Hz, 4-H), 2.63-2.56 (m, 1 H, 6'-H_{exo}), 2.05-1.95 (m, 2 H, 6'-H_{endo}, 5'-H_{exo}), 1.77-1.70 (m, 1 H, 5'$_{endo}$), 1.15 (s, 3 H, 10'-CH_3), 1.11 (s, 3 H, 9'-CH_3), 1.02 (s, 3 H, 8'-CH_3); MS (EI) 435 (MH^+, 1); exact mass 416.1598 ($M^+ - H_2O$) (416.1583 calcd for $C_{21}H_{24}N_2O_7$) .

(2*S*,3*R*)-2-[[(*o*-Nitrophenyl)sulfenyl]amino]-3-hydroxy-4-(*p*-nitrophenyl)butanoic Acid (60).

To a vigorously stirred solution of the amino acid **6** (497 mg, 2.07 mmol) in 1 N NaOH (2.3 mL) and dioxane (5 mL) was added (*o*-nitrophenyl)sulfenyl chloride in small portions while 1 N NaOH solution was added to maintain the reaction mixture at pH 8-9. After an additional 20 min, the reaction mixture was diluted with water (10 mL), acidified with 10% $KHSO_4$ to pH 2.5, and immediately extracted with EtOAc (3 x 15 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated *in*

vacuo. The residue was purified by flash chromatography (1% AcOH/EtOAc) to afford the *N*-protected derivative **60** (736 mg) as an oil in 90 % yield with the following properties: IR (KBr disk) 3421 (br), 1714, 1593, 1514, 1346, 1305, 735 cm^{-1}: ^1H NMR (CD_3OD, 400 MHz) δ 8.27 (m, 2 H, Ar*H*), 8.15 (d, 2 H, *J* = 10 Hz, Ar*H*), 7.74 (m, 1 H, Ar*H*), 7.52 (d, 2 H, *J* = 10 Hz, Ar*H*), 7.33 (m, 1 H, Ar*H*), 4.27 (m, 1 H, 3-H), 3.50 (d, 1 H, *J* = 5 Hz, 2-H), 3.14 (ABX system, 2 H, J_{AB} = 15 Hz, J_{AX} = 5 Hz, J_{BX} = 12 Hz, 4-H); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 43.7 (t), 72.4 (d), 76.8 (d), 126.7 (d), 128.3 (d), 128.7 (d), 128.9 (d), 134.0 (d), 131.3 (d), 146.3 (s), 149.3 (s), 150.4 (s), 150.9 (s), 177.6 (s); MS (FAB+, glycerol) 394 (MH^+).

(3*S*,4*R*)-3-[[(*o*-Nitrophenyl)sulfenyl]amino]-4-[(*p*-nitrophenyl)-methyl]-2-oxetanone (61).

A solution of 4-bromophenylsulfonyl chloride (731 mg, 2.86 mL) in pyridine (4.0 mL) at 0 °C was added dropwise to a solution of **60** (450 mg, 1.14 mmol) in pyridine (4.0 mL) at -45 °C. The reaction mixture was stirred at -45 °C for 1 h and then at 0 °C for 4 h. Crushed ice (ca. 100 mL) was added, and the mixture was acidified with concentrated HCl to pH 2 with vigorous stirring. The mixture was then immediately extracted with EtOAc (3 x 50 mL), and the combined extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the residue by flash chromatography (30 % EtOAc/hexane) gave **61** (100 mg, 24 %) as an oil: IR ($CHCl_3$ cast) 3370 (br), 1822, 1513, 1344, 737 cm^{-1}; ^1H NMR (CD_3CN, 400 MHz) δ 8.28 (m, 1 H, *o*-NO_2-Ar*H*), 8.20 (d, 1 H, *J* = 9 Hz, *p*-NO_2-Ar*H*), 8.05 (m, 1 H, *o*-NO_2-Ar*H*), 7.78 (m, 1 H, *o*-NO_2-Ar*H*), 7.56 (d, 1 H, *J* = 9 Hz, *p*-NO_2-Ar*H*), 7.39 (m, 1 H, *o*-NO_2-Ar*H*), 4.95 (m, 2 H, 3-H, 4-H), 4.51 (d, 1 H, *J* = 9 Hz, N*H*), 3.33 (m, 2 H, CH_2Ar); MS (CI, NH_3) 349 (MNH_4^+ - CO_2, 23), 331 (M^+ - CO_2, 13).

(3*S*,4*R*)-3-Amino-4-[(*p*-nitrophenyl)methyl]-2-oxetanone *p*-Toluenesulfonate Salt (62).

To a stirred suspension of **61** (100 mg, 0.27 mmol) in CH_2Cl_2 (3.0 mL) under argon was added anhydrous *p*-toluenesulfonic acid (48 mg, 0.28 mmol) and *p*-thiocresol (66 mg, 0.53 mmol). The reaction mixture was stirred at 20 °C for 4.5 h, the solvent was evaporated *in vacuo*, and the residue was triturated with diethyl ether until the washings were colorless. The residue was dried under high vacuum to give **62** (71 mg, 68%) as a powder: mp 174 °C (dec.); $[\alpha]_D$ +59.2 °, (c = 0.5, DMF); IR (KBr disk) 3436 (br), 1831, 1519, 1202 cm^{-1}; ^1H NMR (400 MHz, DMF-d_7) δ 8.27, 7.65 (2 x d, 4 H, *J* = 11 Hz, NO_2ArH), 7.68, 7.15 (2 x d, 4 H, *J* = 9 Hz, SO_3ArH), 5.67 (d, 1 H, *J* = 7 Hz, 3-H), 5.33 (m, 1 H, 4-H), 3.66 (m, 2 H, CH_2-$C_6H_4NO_2$), 2.30 (s, 3 H, $ArCH_3$); MS (FAB+, glycerol) 395 (MH^+).

2,3-Dioxosulfinylbenzoyl Chloride (63).

The literature procedure[170] was modified. A suspension of 2,3-dihydroxybenzoic acid (1.00 g, 6.49 mmol) in thionyl chloride (4 mL) was heated to reflux for 3 h to give a clear solution. The excess thionyl chloride was removed *in vacuo* to give an off-white solid which was sublimed (70 °C, 5 mm Hg) to afford **63** (0.54 g, 38%) as fine white crystals. This product was stable at -20 °C for several weeks: mp 78-80 °C (lit.[170] mp 84-86 °C); IR (CH_2Cl_2 cast) 3078, 1740, 1614, 1443, 1270, 1252, 1231, 1187, 1067, 1033, 849, 784, 639 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.94 (dd, 1 H, *J* = 1.2, 8.0 Hz, 6-H), 7.53 (dd, 1 H, *J* = 1.2, 8.0 Hz, 4-H), 7.36 (t, 1 H, *J* = 8 Hz, 5-H); exact mass 217.9435 (217.9440 calcd for $C_7H_3{}^{35}ClO_4S$), MS (CI) 219 (MH^+, ^{35}Cl). Anal. Calcd for $C_7H_3ClO_4S$: C, 38.46; H, 1.38; Cl, 16.22; O, 29.27; S, 14.67. Found: C, 38.62; H, 1.28; Cl, 16.07; O, 29.58; S, 14.74.

(2*S*,3*R*)-2-[(2,3-Dihydroxybenzoyl)amino]-3-hydroxy-4-(*p*-nitrophenyl)butanoic Acid (64)*:

This compound was obtained during the repurification of partially decomposed obafluorin sample by HPLC (reverse phase, R-18): IR (KBr disk) 3420 (br), 1727, 1643, 1518, 1347 cm^{-1}; ^1H NMR (400 MHz, acetone-d_6) δ 8.17 (d, 2 H, J = 9 Hz, O_2N-Ar*H*), 7.96 (br s, 1 H, N*H*), 7.60 (d, 2 H, J = 9 Hz, O_2N-Ar*H*), 7.43 (m, 1 H, Ar*H*), 7.01 (m, 1 H, Ar*H*), 6.79 (m, 1 H, Ar*H*), 4.83, 4.62 (2 x br m, 2 H, C*H*CO_2H, C*H*(OH)), 3.17-3.00 (m, 2 H, ArC*H*$_2$); exact mass 376.0941 (M^+, 3) (376.0907 calcd for $C_{17}H_{16}N_2O_8$).

Hydrolysis and derivatization of 64.*

The hydroxy acid **64** (0.9 mg) was heated to reflux in aqueous hydrochloric acid (6 N, 1 mL) for 22 h. The reaction mixture was concentrated *in vacuo* and purified by ion exchange chromatography on AG 50W-X8 (H^+) resin (0.5 cm x 4 cm) by elution with water followed with 1 N NH_4OH solution. The ammonia washings were lyophilized and the residue was derivatized using (*S*)-camphanic acid chloride and diazomethane as described above for **6** and **57**. ^1H NMR ($CDCl_3$, 500 MHz) analysis produced identical data to the derivative prepared from amino acid **6**.

(3*S*,4*R*)-3-(Acetylamino)-4-(*p*-nitrophenyl)methyl-2-oxetanone (65).*

A suspension of **62** (10.0 mg, 0.025 mmol) in CH_2Cl_2 (0.5 mL) under Ar was cooled to -10 °C and treated with pyridine (4 μL, 0.049 mmol) and acetyl chloride (2.7 μL, 0.038 mmol). The reaction mixture was stirred at - 10 °C for 30 min and allowed to warm to room temperature overnight. The mixture was diluted with EtOAc (5 mL) and washed with water (5 x 5 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated *in vacuo*. The solid residue was triturated with ether (3 x 5 mL) to afford **65** (4.6 mg, 66% yield) as a white powder: IR (KBr disk) 3280, 3600-2800 (br),

1850, 1833, 1822, 1666, 1598, 1546, 1515, 1347, 1340, 1137, 905, 854 cm^{-1}; ^1H NMR (500 MHz, $(CD_3)_2CO$) δ 8.21 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 8.16 (br d, 1 H, *J* = 8.0 Hz, N*H*), 7.60 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 5.76 (dd, 1 H, *J* = 6.0, 8.5 Hz, 3-H), 5.05 (m, 1 H, 4-H), 3.38-3.25 (ABX system, 2 H, J_{AB} = 15 Hz, J_{AX} = 9 Hz, J_{BX} = 5 Hz, O_2N-ArCH_2), 1.99 (s, 3 H, CH_3CO); exact mass 246.0638 (M^+ - H_2O, 6) (246.0641 calcd for $C_{12}H_{10}N_2O_4$), 220.0846 (M^+ - CO_2, 37) (220.0848 calcd for $C_{11}H_{12}N_2O_3$), MS (CI) 265 (MH^+, 69).

(3*S*,4*R*)-3-(Benzoylamino)-4-(*p*-nitrophenylmethyl)-2-oxetanone (66).*

To a suspension of **62** (10.0 mg, 0.025 mmol) in CH_2Cl_2 (0.5 mL) at - 6 °C under Ar were added benzoyl chloride (4.5 μL, 0.038 mmol) and pyridine (4 μL, 0.049 mmol). The reaction mixture was stirred at - 6 °C for 30 min and allowed to warm to room temperature overnight. The mixture was diluted with EtOAc (5 mL) and washed with water (5 x 5 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated *in vacuo*. The solid residue was triturated with ether (2 x 5 mL) to afford **66** (6.7 mg, 81%) as a white powder: IR (KBr disk) 3266, 3600-2800 (br), 1841, 1649, 1537, 1515, 1342 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ 8.78 (d, 1 H, *J* = 8.0 Hz, N*H*), 8.17 (d, 2 H, *J* = 8.0 Hz, O_2NAr*H*), 7.95 (d, 2 H, *J* = 8.0 Hz, Ar*H*CO), 7.60 (m, 3 H, Ar*H*), 7.50 (m, 2 H, Ar*H*CO), 6.0 (dd, 1 H, *J* = 6.0, 8.0 Hz, 3-H), 5.18 (m, 1 H, 4-H), 3.51 (dd, 1 H, *J* = 10, 16 Hz, O_2NArC*H*H), 3.38 (dd, 1 H, *J* = 4, 16 Hz, O_2NArCH*H*); exact mass 281.0925 (M^+ - CO_2H, 20) (281.0925 calcd for $C_{16}H_{13}N_2O_3$), MS (CI, NH_3) 343 (MNH_3^+).

(3*S*,4*R*)-3-[[2-(2-Aminothiazo-4-yl)-2-(methoxyimino)]acetyl]-amino]-4-(*p*-nitrophenylmethyl)-2-oxetanone (67).*

To a stirred solution of 2-amino-α-(methoxyimino)-4-thiazoleacetic acid (7.0 mg, 0.035 mmol) and **62** (12.0 mg, 0.030 mmol) in DMF (0.1 mL) at 0 °C were added diethylphosphoryl cyanide (5.0 μL, 0.033 mmol) and triethylamine (5.0 μL, 0.037 mmol) over 5 min. The mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. The mixture was diluted with EtOAc (2 mL)/benzene (1 mL) and washed with water (1.5 mL) and saturated NaCl solution (2 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification with preparative TLC provides **67** (3.0 mg, 25%): ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 8.21 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 7.63 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 6.87 (s, 1 H, 4-thiazolyl H), 6.52 (br d, 1 H, *J* = 8.0 Hz, N*H*CO), 5.95 (m, 1 H, 3-H), 5.14 (m, 1 H, 4-H), 3.89 (s, 3 H, N-OC*H*$_3$), 3.40 (2 x s, 2 H, O_2NArC*H*$_2$).

(3*S*,4*R*)-3-Amino-4-benzyl-2-oxetanone *p*-Toluenesulfonate Salt (68).

To a stirred suspension of **85** (32.6 mg, 0.099 mmol) in CH_2Cl_2 (2 mL) under argon was added *p*-toluenesulfonic acid (20.0 mg, 0.10 mmol) and *p*-thiocresol (26.8 mg, 0.20 mmol). The reaction mixture was stirred at 20 °C overnight, the solvent was evaporated, and the residue was triturated with diethyl ether until the washings were colorless. The residue was dissolved in the minimum amount of methanol and filtered to remove solid impurities. The filtrate was concentrated to give **68** (31.3 mg, 91%) as a white solid: IR (KBr disk) 3440, 3600-2500 (br), 1830, 1618, 1208, 1167, 1126, 1039, 1014, 814, 680, 569 cm^{-1}; ^{1}H NMR (200 MHz, DMF-d_7) δ 7.67 (d, 2 H, *J* = 8.0 Hz, SO_3-Ar*H*), 7.31 (m, 5 H, Ar*H*), 7.17 (d, 2 H, *J* = 8.0 Hz, SO_3-Ar*H*), 5.64 (d, 1 H, *J* = 7.0 Hz, 3-H), 5.22 (m, 1 H, 4-H), 3.46 (m, 2 H, -C*H*$_2$C$_6$H$_5$), 2.33 (s, 3 H, ArC*H*$_3$); MS (FAB) 350 (MH^+).

(3*S*,4*R*)-3-Amino-4-[(*p*-chlorophenyl)methyl]-2-oxetanone *p*-Toluenesulfonate Salt (69).

To a stirred suspension of **86** (51.0 mg, 0.14 mmol) in CH_2Cl_2 (2 mL) under argon was added *p*-toluenesulfonic acid (29.3 mg, 0.15 mmol) and *p*-thiocresol (34.5 mg, 0.28 mmol). The reaction mixture was stirred at 20 °C for 10 h, the solvent was evaporated, and the residue was triturated with diethyl ether until the washings were colorless. The residue was left under high vacuum to give **69** (53.6 mg, quantitative) as a white solid: mp 158-162 °C (dec.); IR (KBr disk) 3425, 3500-2500 (br), 1830, 1518, 1494, 1337, 1205, 1167, 1154, 1123, 1036, 1012, 814, 800, 680, 569 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ, 7.71 (d, 2 H, *J* = 8.0 Hz, SO_3-Ar*H*), 7.18-7.40 (m, 6 H, Ar*H*), 5.23 (d, 1 H, *J* = 6.0 Hz, 3-H), 5.00 (m, 1 H, 4-H), 3.15 (m, 2 H, CH_2-C_6H_4Cl), 2.36 (s, 3H, SO_3-ArCH_3); MS (FAB+, glycerol) 384 (MH^+, ^{35}Cl).

(3*S*,4*R*)-3-Amino-4-[(*p*-methoxyphenyl)methyl]-2-oxetanone *p*-Toluenesulfonate Salt (70).

To a stirred suspension of **87** (20.1 mg, 0.056 mmol) in CH_2Cl_2 (1.5 mL) under argon was added *p*-toluenesulfonic acid (11.4 mg, 0.060 mmol) and *p*-thiocresol (13.9 mg, 0.11 mmol). The reaction mixture was stirred at 20 °C for 7 h, the solvent was evaporated, and the residue was triturated with diethyl ether until the washings were colorless. The residue was concentrated under high vacuum to afford **70** (17.8 mg, 84%) as a white solid: mp 143-45 °C (dec.); IR (KBr disk) 3424, 3153, 2953, 2924, 2854, 1827, 1516, 1462, 1252, 1206, 1180, 1151, 1124, 1036, 1012, 806, 680, 569 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ, 7.71 (d, 2 H, *J* = 8.0 Hz, SO_3Ar*H*), 7.22, 7.16 (2 x d, 4 H, *J* = 8.0 Hz, SO_3-Ar*H*, CH_3O-Ar*H*), 6.88 (d, 2 H, *J* = 8.0 Hz, CH_3O-Ar*H*), 5.20 (d, 1 H, *J* = 7.0 Hz, 3-H), 4.97 (m, 1 H, 4-H), 3.77 (s, 3 H, CH_3OAr), 3.10 (m, 2 H, $CH_2C_6H_4OCH_3$), 2.35 (s, 3 H, ArCH_3); MS (FAB) 380 (MH^+).

IR Study on the Stability of 70.

A solution of the β-lactone tosylate **70** (1.61 mg, 4.24 x 10^{-3} mmol) in a mixed solvent (THF/water, 3 : 7, 0.3 mL) was prepared and immediately transfered to a 0.1 mm IR-Trans cell (Kodak, polycrystalline ZnS); the β-lactone carbonyl absorption at 1843 cm^{-1} was monitored by the continuous scanning (per scan/10 sec) on a Nicolet 7199 FT-IR spectrometer. The pure solvent was used as the background reference.

The molar extinction coefficient ε at the initial concentration was calculated following the Beer's law: $\mathbf{A = lg\ (I_0/I_t) = \varepsilon lc}$, where **A** is the absorbance, **l** is the width of the cell, and **c** is the molar concentration of the sample. At the initial concentration (**c** = 0.014 M), **A** was measured to be 0.1026, and **l** is a constant, 0.2 mm or 0.02 dm. Therefore, ε is calculated to be 364 dm^2/mol.

The half life-time ($t_{1/2}$) of **70** at which point the absorbance, and hence the concentration decreased to 50 % of the initial value was estimated to be 2 h. This value is similar to that of the previously studied serine β-lactone salt.[176]

***p*-Methoxyphenylacetaldehyde (71).**

The literature method[175] was modified. To a suspension of mercury(II) oxide (4.40 g, 20.3 mmol) in *p*-methoxystyrene (2.00 g, 14.9 mmol), diethyl ether (20 mL), and water (2 mL), was added iodine (5.00 g, 19.7 mmol) in small portions over 1 h. The mixture was filtered and the filtrate was washed twice with saturated sodium thiosulfate solution (2 x 10 mL) and then dried over Na_2SO_4. Evaporation of the solvent *in vacuo* yielded compound **71** (2.23 mg, quantitative) as a liquid. IR (CH_2Cl_2 cast) 3000, 2954, 2935, 2836, 1723, 1611, 1584, 1513, 1464, 1302, 1248, 1178, 1033, 826 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.71(t, 1 H, *J* = 2.4 Hz, C*H*O), 7.12 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 6.91 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 3.80 (s, 3H, CH_3OAr), 3.62 (d, 2 H, *J* = 2.4 Hz, ArCH_2CHO); exact mass 150.0682 (150.0681 calcd for $C_9H_{10}O_2$).

(2*S*,5*S*,1'*R*)-1-Benzoyl-2-(*tert*-butyl)-5-(1'-hydroxy-2'-phenylethyl)-3-methylimidazolidin-4-one (72) and (2*R*,5*S*,1'*R*)-5-(1'-Benzoyloxy-2'-phenylethyl)-2-(*tert*-butyl)-3-methylimidazolidin-4-one (73).

To hexamethyldisilazane (0.50 mL, 2.0 mmol) in THF (2.0 mL) at -78 °C was added *n*-butyllithium (0.70 M in hexanes, 3.40 mL, 2.4 mmol). The solution was stirred at -78 °C for 1 h, then at 20 °C for 5 min. The solvent was removed under high vacuum and the resulting white powder was cooled to -78 °C and redissolved in THF (20 mL); to this solution was slowly added a solution of (*S*)-1-benzoyl-2-(*tert*-butyl)-3-methyl-4-imidazolidinone (**14**) (0.58 g, 2.2 mmol) in THF (10 mL), and the resulting orange/red solution was stirred at -78 °C for 30 min. A solution of phenylacetaldehyde (0.38 g, 3.2 mmol) in THF (10 mL) was added dropwise over 20 min. The mixture was stirred at -78 °C for 30 min, and then allowed to warm to room temperature over 1 h. The reaction was quenched by the addition of a saturated aqueous NH_4Cl solution (20 mL) with shaking. The mixture was extracted with diethyl ether (3 x 25 mL). The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was purified by flash chromatography (40% EtOAc/hexane) to give a mixture of the expected aldol adduct **72** and the rearranged isomer **73** (**72/73**, 60 : 40) (0.42 g, 55%) as a foam: IR ($CHCl_3$ cast) 3380 (br), 2960, 1716, 1692, 1452, 1270, 1111, 1070, 1027, 752, 701 cm^{-1}; ^1H NMR for **72** (200 MHz, $CDCl_3$) δ 7.75 (d, 2 H, *J* = 7.0 Hz, Ar*H*), 7.50 (m, 6 H, Ar*H*), 7.10 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 5.78 (br s, 1 H, 2-H), 4.65 (m, 2 H, 5-H and O*H*), 3.45 (m, 1 H, 1'-H), 3.16 (s, 3 H, CH_3N), 2.65 (d, 1 H, *J* = 12 Hz, ArC*H*H), 2.32 (m, 1 H, ArCH*H*), 1.09 (s, 9 H, C(CH_3)$_3$); ^1H NMR for **73** (200 MHz, $CDCl_3$) δ 8.14-7.95 (m, 6 H, Ar*H*CO), 7.50 (m, 4 H, Ar*H*), 5.50 (m, 1 H, 1'-H), 4.01 (d, 1 H, *J* = 2 Hz, 2-H), 3.89 (m, 1 H, H-5), 3.29 (d, 2 H, *J* = 10 Hz, ArCH_2), 2.90 (s, 3 H, CH_3N), 0.89 (s, 9 H, C(CH_3)$_3$); exact mass 323.1398 (M^+ - *t*-Bu, 49) (323.1395 calcd for $C_{19}H_{19}N_2O_3$), MS (CI) 381 (MH^+, 100).

(2*S*,5*S*,1'*R*)-1-Benzoyl-2-(*tert*-butyl)-5-[1'-hydroxy-2'-(*p*-chlorophenyl)ethyl]-3-methylimidazolidin-4-one (74) and (2*R*,5*S*,1'*R*)-5-[1'-Benzoyloxy-2'-(*p*-chlorophenyl)ethyl]-2-(*tert*-butyl)-3-methylimidazolidin-4-one (75).

To hexamethyldisilazane (1.50 mL, 7.20 mmol) in THF (4.0 mL) at -78 °C was added *n*-butyllithium (1.95 M in hexanes, 3.70 mL, 7.20 mmol). The mixture was stirred at -78 °C for 10 min, then at 20 °C for 5 min to give a clear solution. The solution was cooled to -78 °C, and a solution of (*S*)-1-benzoyl-2-(*tert*-butyl)-3-methyl-4-imidazolidinone (**14**) (1.56 g, 6.00 mmol) in THF (50 mL) was slowly added over 30 min. The resulting orange/red solution was stirred at -78 °C for 1 h. To the enolate solution was added dropwise a solution of (*p*-chlorophenyl)acetaldehyde (**54**) (1.66 g, 10.7 mmol) in THF (30 mL) over 20 min. After stirring at -78 °C for 1.5 h, the reaction was quenched by the addition of a saturated aqueous NH_4Cl solution (100 mL) with vigorous stirring, and the mixture was allowed to warm to room temperature over ca. 30 min. The mixture was extracted with diethyl ether (3 x 80 mL), and the combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo* to give a foam (2.75 g). The crude product was purified by flash chromatography (40% EtOAc/hexane) to give a white solid (1.43 g, 57%). This product was deduced by ^1H NMR to be a mixture of the expected aldol adduct **74** and the rearranged isomer **75** in a 55:45 ratio. The ^1H NMR assignments were based on proton decoupling studies and by comparison with the literature data of similar compounds: mp 143-145 °C; IR (KBr) 3400 (br), 2930, 1680, 1635, 1492,1447, 1405, 1385, 1362, 1260, 1085, 1015 cm^{-1}; ^1H NMR of **74** (400 MHz, $CDCl_3$) δ 7.72 (d, 2 H, *J* = 7.0 Hz, Ar*H*CO), 7.44 (m, 3 H, Ar*H*CO), 7.15 (d, 2 H, *J* = 8.1 Hz, Cl-Ar*H*), 6.89 (d, 2 H, *J* = 8.1 Hz, Cl-Ar*H*), 5.74 (br s, 1 H, 2-H), 4.59 (d, 1 H, *J* = 4.2 Hz, 5-H), 4.51 (d, 1 H, *J* = 11.8 Hz, O*H*), 3.38 (m, 1 H, 1'-H), 3.11 (s, 3 H, CH_3N), 2.53 (d, 1 H, *J* = 14.4 Hz, Cl-ArC*H*H), 2.21 (m, 1 H, Cl-ArCH*H*), 1.09 (s, 9 H, C(CH_3)$_3$); ^1H NMR of **75**

(400 MHz, $CDCl_3$) δ 7.95 (d, 2 H, J = 7.2 Hz, Ar*H*CO), 7.54 (m, 3 H, Ar*H*CO), 7.25 (m, 4 H, Cl-Ar*H*), 5.52 (m, 1 H, 1'-H), 4.22 (m, 1 H, 2-H), 3.68 (m, 1 H, 5-H), 3.20 (m, 2 H, Cl-ArCH_2), 2.91 (s, 3 H, CH_3N), 0.98 (s, 9H, C(CH_3)$_3$); exact mass 359.0990 (M^+ - *t*-Bu, 7) (359.0986 calcd for $C_{19}H_{18}N_2O_3{}^{37}Cl$), 357.1015 ($M^+$ - *t*-Bu, 7) (357.1006 calcd for $C_{19}H_{18}N_2O_3{}^{35}Cl$).

(2*S*,5*S*,1'*R*)-1-Benzoyl-2-(*tert*-butyl)-5-[1'-hydroxy-2'-(*p*-methoxyphenyl)ethyl]-3-methylimidazolidin-4-one (76) and (2*R*,5*S*,1'*R*)-5-[1'-Benzoyloxy-2'-(*p*-methoxyphenyl)ethyl]-2-(*tert*-butyl)-3-methylimidazolidin-4-one (77).

To hexamethyldisilazane (2.50 mL, 12.0 mmol) in THF (7.0 mL) at -78 °C was added *n*-butyllithium (1.53 M in hexanes, 7.80 mL, 12.0 mmol). The solution was stirred at -78 °C for 10 min, then at 20 °C for 5 min. The solution was recooled to -78 °C and a solution of (*S*)-1-benzoyl-2-(*tert*-butyl)-3-methyl-4-imidazolidinone (**14**) (2.60 g, 10.0 mmol) in THF (60 mL) was slowly added over 30 min, and the stirring was continued at -78 °C for an additional 30 min. To the enolate solution was added dropwise a solution of *p*-methoxyphenylacetaldehyde (**71**) (2.30 g, 16.0 mmol) in THF (15 mL) over 20 min. After stirring at -78 °C for 1.5 h, the reaction was slowly warmed to room temperature over 1 h and quenched by the addition of a saturated aqueous NH_4Cl solution (100 mL). The aqueous layer was extracted with diethyl ether (3 x 80 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash chromatography (40% EtOAc/hexane) to give a white solid (1.70 g, 41%). This product was deduced by ^1H NMR to be a mixture of the expected aldol adduct **76** and the rearranged isomer **77** in 72:28 ratio. The ^1H NMR assignments were based on proton decoupling studies and by comparison with the literature data: mp 155-158 °C; IR (KBr) 3413, 2958, 1717, 1683, 1632, 1615, 1514, 1407, 1389, 1250, 1179, 1082, 1029 cm^{-1}; ^1H NMR of **76**

(400 MHz, $CDCl_3$) δ 7.73 (d, 2 H, J = 7.1 Hz, Ar*H*CO), 7.49 (m, 3 H, Ar*H*CO), 6.88 (d, 2 H, J = 8.4 Hz, CH_3O-Ar*H*), 6.74 (d, 2 H, J = 8.4 Hz, CH_3O-Ar*H*), 5.74 (br s, 1 H, 2-H), 4.59 (d, 1 H, J = 4.4 Hz, 5-H), 4.42 (d, 1 H, J = 10.5 Hz, O*H*), 3.74 (s, 3 H, C*H*$_3$OAr), 3.39 (m, 1H, 1'-H), 3.10 (s, 3 H, C*H*$_3$N), 2.53 (d, 1 H, J = 14.1 Hz, CH_3O-ArC*H*H), 2.07 (m, 1 H, CH_3O-ArCH*H*), 1.09 (s, 9 H, C(C*H*$_3$)$_3$); ^{1}H NMR of **77** (400 MHz, $CDCl_3$) δ 7.95 (m, 2 H, Ar*H*CO), 7.49 (m, 3H, Ar*H*CO), 7.25 (d, 1 H, J = 8.0 Hz, N*H*), 5.52 (m, 1 H, 1'-H), 4.20 (m, 1 H, 2-H), 3.70 (m, 1 H, 5-H), 3.15 (m, 2 H, CH_3O-ArC*H*$_2$), 2.91 (s, 3 H, C*H*$_3$N), 0.98 (s, 9 H, C(C*H*$_3$)$_3$); MS (CI) 411 (MH^+, 100). Anal. Calcd for $C_{24}H_{30}N_2O_4$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.25; H, 7.15; N, 6.67.

(2*S*,3*R*)-2-Amino-3-hydroxy-4-phenylbutanoic Acid (78).

A suspension of a mixture of **72** and **73** (991 mg, 2.60 mmol) in 6 N HCl (40 mL) was heated at reflux overnight. The yellow solution was cooled to room temperature, extracted with Et_2O (3 x 40 mL), and concentrated *in vacuo* to 5 mL. This residue was applied to an AG 50W-X8 (H^+) ion-exchange column which was then eluted with water followed by 1 N NH_4OH solution. The fractions containing the desired amino acid (ninhydrin positive) were first concentrated *in vacuo* to remove ammonia and then lyophilized to yield **78** (0.40 g, 78%) as a white solid: mp 195-198 °C; IR (KBr) 3418 (s), 3600-2200 (br), 1617, 1602, 1585, 1577, 1507, 1496, 1407, 1335, 1077, 697 cm^{-1}; ^{1}H NMR (200 MHz, D_2O + DCl) δ 7.20 (m, 5 H, Ar*H*), 4.16 (m, 1 H, 3-H), 3.50 (d, 1 H, J = 4.5 Hz, 2-H), 2.89 (dd, 1 H, J = 5.0, 14 Hz, ArC*H*H), 2.68 (dd, 1 H, J = 10, 14 Hz, ArCH*H*); MS (CI) 196 (MH^+, 100). Anal. Calcd for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.17. Found: C, 61.42; H, 6.76; N, 7.15.

(2*S*,3*R*)-2-Amino-3-hydroxy-4-(*p*-chlorophenyl)butanoic Acid (79).

A suspension of the mixture of **74** and **75** (1.30 g, 3.13 mmol) in 6 N HCl (40 mL) was heated at reflux overnight. The yellow solution was cooled at room temperature, extracted with Et_2O (3 x 60 mL) and concentrated *in vacuo*. The residue was applied to an AG 50W-X8 (H^+) ion-exchange column and eluted with water followed by 1N NH_4OH solution. The fractions containing the desired amino acid (ninhydrin positive) were first concentrated *in vacuo* to remove ammonia and then lyophilized to yield **79** (0.46 g, 64%) as a white solid: mp 202-205 °C; IR (KBr) 3422 (s), 3700-2800 (br), 1656, 1641, 1632, 1613, 1599, 1535, 1408 cm^{-1}; ^1H NMR (200 MHz, D_2O + DCl) δ 6.70 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 6.62 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 3.80 (m, 1 H, 3-H), 3.47 (d, 1 H, *J* = 4.5 Hz, 2-H), 2.36 (dd, 1 H, *J* = 5.0, 15 Hz, ArC*H*H), 2.18 (dd, 1 H, *J* = 10, 15 Hz, ArCH*H*); exact mass 184.0530 (M^+ - CO_2H) (184.0529 calcd for $C_9H_{11}ClNO$). Anal. Calcd for $C_{10}H_{12}ClNO_3$: C, 52.30; H, 5.27; N, 6.10. Found: C, 52.17; H, 5.16; N, 5.96.

(2*S*,3*R*)-2-Amino-3-hydroxy-4-(*p*-hydroxyphenyl)butanoic Acid (80).

A suspension of the mixture of **76** and **77** (1.50 g, 3.65 mmol) in 6 N HCl solution (40 mL) was heated to reflux overnight. The yellow solution was cooled to room temperature, diluted with water (80 mL), extracted with Et_2O (3 x 60 mL), and concentrated *in vacuo* to give a brown solid. This residue was purified on an AG 50W-X8 (H^+) ion-exchange column which was eluted with water followed by 1 N NH_4OH solution. The fractions containing the desired amino acid were first concentrated *in vacuo* to remove ammonia and then lyophilized to yield **80** (770 mg, quantitative) as a solid: mp 214-216 °C (dec.); IR (KBr) 3360, 3140, 3045, 3650-2500 (br), 1637, 1613, 1515, 1403, 1240 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 7.06 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 6.74 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 4.14 (m, 1 H, 3-H), 3.53 (d, 1 H, *J* = 4.5 Hz,

2-H), 2.80 (dd, 1 H, J = 5.0, 14 Hz, ArC*H*H), 2.62 (dd, 1 H, J = 10, 14 Hz, ArCH*H*); MS (FAB, glycerol) 212.08 (MH^+) (212.09 calcd for $C_{10}H_{14}NO_4$).

(2*S*,3*R*)-2-Amino-3-hydroxy-4-(*p*-methoxyphenyl)butanoic Acid (81).

A suspension of the mixture of **76** and **77** (1.48 g, 3.60 mmol) in 6 N HCl solution (30 mL) was heated at reflux for 5.5 h. The yellow solution was cooled to room temperature, diluted to 60 mL with water, extracted with Et_2O (3 x 60 mL), and concentrated *in vacuo* to give a yellow solid (0.98 g). The material was purified on an AG 50W-X8 (H^+) ion-exchange column eluted with water followed by 1N NH_4OH solution. The fractions containing the desired amino acid were first concentrated *in vacuo* to remove ammonia and then lyophilized to yield **81** (0.56 g, 69%) as a white solid: mp 190-191 °C (dec.); IR (KBr) 3437 (s), 3700-2800 (br), 1656, 1631, 1613, 1528, 1408, 1252 cm^{-1}; ^1H NMR (200 MHz, D_2O + DCl) δ 7.13 (d, 2 H, J = 8.0 Hz, Ar*H*), 6.84 (d, 2 H, J = 8.0 Hz, Ar*H*), 4.12 (m, 1 H, 3-H), 3.68 (s, 3 H, C*H*$_3$OAr), 3.52 (d, 1 H, J = 4.5 Hz, 2-H), 2.82 (dd, 1 H, J = 5.0, 14 Hz, ArC*H*H), 2.63 (dd, 1 H, J = 10, 14 Hz, ArCH*H*); MS (FAB) 226.07 (MH^+) (226.10 calcd for $C_{11}H_{16}NO_4$).

(2*S*,3*R*)-2-[[(*o*-Nitrophenyl)sulfenyl]amino]-3-hydroxy-4-phenylbutanoic Acid (82).

To a vigorously stirred solution of **78** (241.0 mg, 1.24 mmol) in 1 N NaOH (2.5 mL) and dioxane (1.0 mL) was added (*o*-nitrophenyl)sulfenyl chloride (260. mg, 1.37 mmol) in small portions while 2 N NaOH solution was added dropwise to keep the reaction mixture around pH 8-9. After an additional 20 min, the reaction mixture was filtered. The filtrate was acidified with 10% $KHSO_4$ to pH 2-3 and extracted with EtOAc (3 x 20 mL). The combined extracts were dried (Na_2SO_4) and concentrated to yield **82** (302 mg, 70%) as a yellow solid: IR (CH_3OH cast) 3600-3200 (br), 1717,

1700, 1507, 1385, 1363, 1338, 1306 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 8.37-8.25 (m, 2 H, Ar*H*), 7.80 (m, 1 H, Ar*H*), 7.38-7.15 (m, 5 H, Ar*H*), 4.26 (m, 1 H, 3-H), 3.43 (d, 1 H, *J* = 3.9 Hz, 2-H), 3.12-2.91 (m, 2 H, ArCH_2); MS (FAB) 349 (MH^+).

(2*S*,3*R*)-2-[[(*o*-Nitrophenyl)sulfenyl]amino]-3-hydroxy-4-(*p*-chlorophenyl)butanoic Acid (83).

To a vigorously stirred solution of **79** (0.40 g, 1.74 mmol) in 2 N NaOH (1.25 mL), water (4.0 mL), and dioxane (1.0 mL) was added in small portions (*o*-nitrophenyl)sulfenyl chloride (363 mg, 1.90 mmol) while 2 N NaOH solution was added dropwise to keep the reaction mixture at pH 8-9. After an additional 30 min, the reaction was quenched by adding water (10 mL) and the mixture was filtered to remove a solid residue. The filtrate was acidified with 10% $KHSO_4$ to pH 2.5 and extracted with EtOAc (3 x 10 mL). The combined extracts were dried (Na_2SO_4) and concentrated to an oil. Purification by flash chromatography (1% AcOH/EtOAc) afforded the *N*-protected amino acid **83** (394 mg, 59%) as a bright yellow solid: mp 68-75 °C; IR (KBr disk) 3434 (br), 1718, 1709, 1636, 1631, 1592, 1508, 1337, 1306 cm^{-1}: ^1H NMR (200MHz, $(CD_3)_2CO$) δ 8.40 (d, 1 H, *J* = 8 Hz, Ar*H*), 8.28 (d, 1 H, *J* = 8 Hz, Ar*H*), 7.81 (t, 1 H, *J* = 8 Hz, Ar*H*), 7.34 (m, 5 H, Ar*H*), 4.34 (m, 2 H, 3-H and N*H*), 3.59 (m, 1 H, 2-H), 3.10 (m, 2 H, ArCH_2); MS (FAB) 383 (MH^+).

(2*S*,3*R*)-2-[[(*o*-Nitrophenyl)sulfenyl]amino]-3-hydroxy-4-(*p*-methoxyphenyl)butanoic Acid (84).

To a vigorously stirred solution of **81** (600 mg, 2.60 mmol) in 2 N NaOH (2.5 mL), water (2.0 mL), and dioxane (2.0 mL), was added (*o*-nitrophenyl)sulfenyl chloride (542 mg, 2.86 mmol) in small portions while 2 N NaOH solution was added

dropwise to keep the pH 8-9. After an additional 20 min, the mixture was diluted with water (20 mL) and filtered. The filtrate was acidified with 10% $KHSO_4$ to pH 2-3 and extracted with EtOAc (3 x 50 mL). The combined extracts were dried (Na_2SO_4) and concentrated to afford **84** (453 mg, 45%): mp 168-170 °C; IR (acetone cast) 3600-2800 (br), 1712, 1592, 1566, 1512, 1337, 1305, 1247 cm^{-1}: ^1H NMR (200 MHz, $(CD_3)_2CO$) δ 8.50 (d, 1 H, *J* = 8.0 Hz, Ar*H*), 8.32 (d, 1 H, *J* = 8 Hz, Ar*H*), 7.88 (t, 1 H, *J* = 8 Hz, Ar*H*), 7.45(t, 1 H, *J* = 8 Hz, Ar*H*), 7.28 (d, 2 H, *J* = 8.5 Hz, Ar*H*), 6.89 (d, 2 H, *J* = 8.5 Hz, Ar*H*), 4.40 (m, 2 H, 3-H and N*H*), 3.80 (s, 3 H, C*H*$_3$OAr), 3.62 (m, 1 H, 2-H), 3.13 (m, 2 H, ArC*H*$_2$); MS (FAB) 379.08 (MH^+) (379.10 calcd for $C_{17}H_{19}N_2O_6S$).

(3*S*,4*R*)-3-[[(*o*-Nitrophenyl)sulfenyl]amino]-4-benzyl-2-oxetanone (85).

A solution of 4-bromophenylsulfonyl chloride (542 mg, 2.13 mmol) in pyridine (3.0 mL) at 0 °C was added dropwise to a solution of **82** (296 mg, 0.85 mmol) in pyridine (3.0 mL) at -45 °C. The reaction mixture was stirred at -45 °C for 1 h and then at 0 °C for 3 h. Crushed ice (ca. 20 mL) was added, and the mixture was acidified with concentrated HCl to pH 2 with vigorous stirring. The mixture was then immediately extracted with EtOAc (3 x 30 mL), and the combined extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give a dark brown solid. Purification of the residue by flash chromatography (15% EtOAc/hexane) gave **85** (116 mg, 41%) as a bright yellow solid: IR ($CHCl_3$, cast) 3365, 1824, 1590, 1567, 1512, 1337, 1306, 740 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.32 (d, 1 H, *J* = 8.0 Hz, *o*-NO_2-Ar*H*), 8.05 (m, 1 H, *o*-NO_2-Ar*H*), 7.76 (m, 1 H, *o*-NO_2-Ar*H*), 7.35 (m, 6 H, *o*-NO_2-Ar*H* and Ar*H*), 4.79-5.08 (m, 2 H, 3-H, 4-H), 3.10-3.55 (m, 3 H, N*H* and C*H*$_2$Ar); MS (FAB) 331 (MH^+).

(3*S*,4*R*)-3-[[(*o*-Nitrophenyl)sulfenyl]amino]-4-[(*p*-chlorophenyl)-methyl]-2-oxetanone (86).

A solution of 4-bromophenylsulfonyl chloride (594 mg, 2.30 mmol) in pyridine (3.2 mL) at 0 °C was added dropwise to a solution of **83** (357 mg, 0.93 mmol) in pyridine (3.3 mL) at -45 °C. The mixture was stirred at -45 °C for 1 h, then at 0 °C for 2 h. The reaction mixture was poured into crushed ice (ca. 50 mL) and acidified with concentrated HCl to pH 2 with vigorous stirring. The mixture was immediately extracted with EtOAc (3 x 20 mL), and the combined extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give an oil. Purification of the residue by flash chromatography (20% EtOAc/hexane) gave **86** (85 mg, 25 %) as a bright yellow solid: mp 148-150 °C; IR (KBr disk) 3368 (br), 1810, 1590, 1567, 1509, 1492, 1328, 1309, 1277, 1178, 1156, 1099, 823, 736 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.35 (dd, 1 H, *J* = 1.2, 8.0 Hz, *o*-NO_2-Ar*H*), 8.03 (dd, 1 H, *J* = 1.2, 8.0 Hz, *o*-NO_2-Ar*H*), 7.76 (m, 1 H, *o*-NO_2-Ar*H*), 7.32 (m, 5 H, *o*-NO_2-Ar*H* and Cl-Ar*H*), 4.84 (m, 2 H, 3-H, 4-H), 3.44 (d, 1 H, *J* = 8.0 Hz, N*H*), 3.19 (m, 2 H, CH_2Ar); MS (FAB) 365 (MH^+, ^{35}Cl), 367 (MH^+, ^{37}Cl).

(3*S*,4*R*)-3-[[(*o*-Nitrophenyl)sulfenyl]amino]-4-[(*p*-methoxy-phenyl)methyl]-2-oxetanone (87).

A solution of 4-bromophenylsulfonyl chloride (321.0 mg, 1.42 mmol) in pyridine (1.5 mL) at 0 °C was added dropwise to a solution of **84** (188.0 mg, 0.57 mmol) in pyridine (1.5 mL) at -45 °C. The reaction mixture was stirred at -45 °C for 1 h, then at 0 °C for 1 h. It was then poured into crushed ice (ca. 20 mL) and acidified with concentrated HCl to pH 2 with vigorous stirring. The mixture was immediately extracted with EtOAc (4 x 10 mL), and the combined extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give an oil. Purification of the residue by flash chromatography (20% EtOAc/hexane) gave **87** (61.7 mg, 34 %) as a bright yellow

solid: mp 67-68 °C; IR ($CHCl_3$ cast) 3366, 1824, 1611, 1593, 1567, 1513, 1338, 1307, 1247, 1179, 736 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.28 (dd, 1 H, *J* = 1.2, 8.0 Hz, *o*-NO_2-Ar*H*), 8.02 (dd, 1 H, *J* = 1.2, 8.0 Hz, *o*-NO_2-Ar*H*), 7.71 (m, 1 H, *o*-NO_2-Ar*H*), 7.32 (m, 1 H, *o*-NO_2-Ar*H*), 7.21 (d, 2 H, *J* = 8.0 Hz, CH_3O-Ar*H*), 6.89 (d, 2 H, *J* = 8.0 Hz, CH_3O-Ar*H*), 4.72-4.92 (m, 2 H, 3-H, 4-H), 3.80 (s, 3 H, C*H*$_3$O-Ar), 3.56 (d, 1 H, *J* = 8.0 Hz, N*H*), 3.19 (m, 2 H, C*H*$_2$Ar); exact mass 360.0785 (360.0780 calcd for $C_{17}H_{16}N_2O_5S$), 316.0880 (M^+ - CO_2, 11) (316.0881 calcd for $C_{16}H_{16}N_2O_3S$). Anal. Calcd for $C_{17}H_{16}N_2O_5S$: C, 56.66; H, 4.48; N, 7.77. Found: C, 56.23; H, 4.40; N, 7.33.

3-((2',3'-Dihydroxybenzoyl)amino)-4-methyl-2-oxetanone (88).

To a suspension of the L-threonine β-lactone tosylate salt **33** (26.1 mg, 0.095 mmol) in CH_2Cl_2 (2 mL) at 0 °C were added acid chloride **63** (22.8 mg, 0.104 mmol) and pyridine (45.2 mg, 0.57 mmol). The reaction mixture was stirred at 0 °C for 30 min and then at room temperature overnight. The solvent was removed *in vacuo* and the residue was triturated with EtOAc (15 mL). The organic phase was washed with water (2 x 10 mL) and 10% $KHSO_4$ solution (10 mL), dried over Na_2SO_4, and concentrated *in vacuo* to afford a solid (21.7 mg). The crude product was purified by flash chromatography (10-30% EtOAc-hexane) to give **88** (8.5 mg, 38%): IR ($CHCl_3$ cast) 3600-2800 (br), 3380, 2922, 1816, 1644, 1541, 1460, 1337, 1270, 1205, 1150, 1125, 746 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.18-6.96 (m, 3 H, N*H*CH, Ar*H*), 6.81 (t, 1 H, *J* = 8 Hz, Ar*H*), 5.75 (dd, 1 H, *J* = 6, 8 Hz, 3-H), 5.02 (quint., 1 H, *J* = 6.0 Hz, 4-H), 1.52 (d, 3 H, *J* = 6.0 Hz, C*H*$_3$CH); exact mass 237.0635 (237.0638 calcd for $C_{11}H_{11}NO_5$), 219.0527 (M^+ - H_2O) (219.0531 calcd for $C_{11}H_9NO_4$).

***tert*-Butyl Ethylenediamineacetate (89).**

The literature procedure[180] was modified. To a solution of NaI (1.12 g, 7.44 mmol) and ethylenediamine (8.88 g, 148 mmol) in DMF (1 mL) at 0 °C was added *tert*-butyl bromoacetate (1.45 g, 7.44 mmol) over 30 min. The reaction mixture was allowed to warm slowly to 5 °C over 2 h. The solvent and excess diamine were removed under high vacuum. The residue was separated by flash chromatography (CH_3OH-CH_2Cl_2, 1:1) to provide **89** (1.28 g, 99%) as a foam: IR (MeOH/$CHCl_3$ cast) 3400, 3160, 3135, 2978, 2932, 1728, 1689, 1460, 1396, 1370, 1270, 1254, 1158 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 3.35 (s, 2 H, $NHCH_2CO$), 2.95 (m, 2 H, $NHCH_2CH_2NH_2$), 2.86 (m, 2 H, $NHCH_2CH_2NH_2$), 1.47 (s, 9 H, $C(CH_3)_3$); MS (CI) MH^+, 175 (100).

Tribenzyl *tert*-Butyl Ethylenediaminetetraacetate (90).

The literature procedure[180] was modified. To a solution of **89** (540 mg, 3.12 mmol) in DMF (12 mL) at 0 °C were added diisopropylethylamine (1.28 g, 9.98 mmol) and benzyl bromoacetate (2.40 g, 9.98 mmol). The reaction mixture was stirred at 0 °C for 30 min and then at 45 °C for 20 h. The solvent was removed under high vacuum, and the residue was dissolved in CH_2Cl_2 (30 mL) and washed sequentially with saturated $NaHCO_3$ solution (30 mL), saturated NaCl solution (30 mL), and water (30 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash chromatography (20% EtOAc/hexane) to afford the EDTA tetra-ester **90** (0.87 g, 45%) as an oil: IR ($CHCl_3$ cast) 2976, 2956, 1743, 1455, 1367, 1257, 1214, 1171, 1152, 996, 739, 697 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.33 (s, 15 H, Ar*H*), 5.11 (s, 6 H, 3 x OCH_2Ph), 3.66 (s, 4 H, $N(CH_2CO_2Bn)_2$), 3.62 (s, 2 H, NCH_2CO_2Bn), 3.45 (s, 2 H, $NCH_2CO_2C(CH_3)_3$), 2.90 (br s, 4 H, NCH_2CH_2N), 1.42 (s, 9 H, $C(CH_3)_3$); exact mass 618.2935

(618.2941 calcd for $C_{35}H_{42}N_2O_8$), 517.2337 (M^+ - $CO_2C(CH_3)$) (517.2338 calcd for $C_{30}H_{33}N_2O_6$).

Ethylenediaminetetraacetic Acid Tribenzyl Ester (91).

The literature procedure[180] was modified. A solution of the EDTA tetra-ester **90** (189 mg, 0.30 mmol) in CH_2Cl_2 (0.5 mL) and trifluoroacetic acid (1 mL) was stirred for 1.5 h at room temperature. The solvent was removed under high vacuum and the residue was redissolved in CH_2Cl_2 (10 mL) and sequentially washed with saturated $NaHCO_3$ solution (10 mL), saturated NaCl solution (10 mL), and water (10 mL). The organic phase was dried (Na_2SO_4) and concentrated *in vacuo* to give **91** (133 mg, 80%) as a sticky oil: IR ($CHCl_3$ cast) 2952, 1740, 1608, 1498, 1455, 1259, 1187, 1137, 996, 739, 697 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.30 (br s, 15 H, Ar*H*), 5.09 (br s, 6 H, 3 x O*CH*$_2$Ph), 3.59 (m, 4 H, N(*CH*$_2$$CO_2$Bn)$_2$), 3.45 (m, 4 H, $HO_2CCH_2NCH_2CO_2Bn$), 2.80 (br s, 4 H, N*CH*$_2$*CH*$_2$N); exact mass 562.2297 (562.2315 calcd for $C_{31}H_{34}N_2O_8$), 517.2339 (M^+ - CO_2H) (517.2338 calcd for $C_{30}H_{33}N_2O_6$).

(3*S*,4*R*)-3-[[*N*-[(Benzyloxycarbonyl)methyl]-*N*-[[2'-[*N*',*N*'-di[(Benzyloxycarbonyl)methyl]amino]ethyl]amino]acetylamino]-4-methyl-2-oxetanone (92).

To a stirred solution of acid **91** (55.6 mg, 0.099 mmol) and the tosylate salt **33** (27.3 mg, 0.10 mmol) in DMF (2 mL) at 0 °C were added diethylphosphoryl cyanide (19.4 mg, 0.11 mmol) and triethylamine (22.3 mg, 0.22 mmol) over 5 min. The resulting deep red solution was stirred at 0 °C for 45 min and then at room temperature for 23 h. The reaction mixture was diluted with EtOAc (20 mL) and washed with water (10 mL). The organic solution was dried over Na_2SO_4 and concentrated under high vacuum. The crude product was dissolved in a minimum volume of $CHCl_3$ and

quickly filtered through a small SiO_2 column. The solvent was removed *in vacuo* and the residue was triturated with hexane to afford **92** (37.3 mg, 58%) as an oil: IR ($CHCl_3$ cast) 3250, 2954, 1823, 1742, 1679, 1185, 1177, 739, 698 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.54 (d, 1 H, *J* = 8 Hz, N*H*), 7.32 (br s, 15 H, Ar*H*), 5.55 (dd, 1 H, *J* = 6.0, 8.0 Hz, C*H*NH), 5.11 (m, 6 H, 3 x PhCH_2O), 4.82 (quint., 1 H, *J* = 6.0 Hz, C*H*CH_3), 3.61 (2 x s, 4 H, N(CH_2CO_2Bn)$_2$), 3.40 (br s, 4H, $BnO_2CCH_2NCH_2CONH$), 2.78 (m, 4 H, NCH_2CH_2N), 1.46 (d, 3 H, *J* = 6.0 Hz, CH_3CH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.1, 171.2 (2 x C), 170.7, 169.2, 135.5, 135.3, 128.5, 128.4, 128.3, 74.8, 66.6, 66.5, 58.6, 58.1, 54.9, 54.8, 52.6, 51.6, 14.6; exact mass 601.2776 (601.2766 calcd for $C_{34}H_{39}N_3O_7$), MS (FAB) 602 (MH^+).

4'-Methyl-2,2'-bipyridine-4-carboxaldehyde (93).

The literature procedure[199] was modified. A suspension of 4,4'-dimethyl-2,2'-bipyridine (5.00 g, 27.1 mmol) and SeO_2 (3.28 g, 29.6 mmol) in 1,4-dioxane (250 mL) was heated under reflux for 24 h and filtered hot. The filtrate was cooled to room temperature and the solvent was removed *in vacuo*. The residue was partially dissolved in ethyl acetate (500 mL) and filtered to remove a dark-brown solid. The filtrate was extracted with 1.0 M Na_2CO_3 solution (2 x 100 mL) to remove the carboxylic acid side products and with 0.3 M $Na_2S_2O_5$ solution (3 x 100 ml) to form an aldehyde-bisulfite adduct. The combined aqueous extracts were adjusted to pH 10 with saturated Na_2CO_3 solution to release the aldehyde and was then extracted with CH_2Cl_2 (4 x 100 mL). The solvent was removed *in vacuo* from the combined organic extracts to afford the monoaldehyde **93** (1.12 g, 21%) as a white solid: mp 123-125 °C (lit.[199] mp 130.9-131.7 °C); IR (KBr) 1704, 1607, 1596, 1558, 1462, 1354, 1293, 1251, 1209, 1150, 834, 752, 668 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 10.22 (s, 1 H, -C*H*O), 8.99 (br s, 1 H, 3-H), 8.93 (d, 1 H, *J* = 5 Hz, 6-H), 8.63 (d, 1 H, *J* = 5.1 Hz, 6'-H), 8.36 (br

s, 1 H, 3'-H), 7.77 (dd, 1 H, J = 1.6, 5.0 Hz, 5-H), 7.29 (d, 1 H, J = 5 Hz, 5'-H), 2.52 (s, 3 H, -CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 191.6, 158.2, 150.3, 149.1, 148.5, 142.5, 125.4, 122.1, 121.4, 120.6, 21.2; exact mass 198.0792 (100) (198.0793 calcd for $C_{12}H_{10}N_2O$). Anal. Calcd for $C_{12}H_{10}N_2O$: C, 72.71; H, 5.08; N, 14.13. Found: C, 72.53; H, 5.04; N, 14.13.

4'-Methyl-2,2'-bipyridine-4-carboxylic Acid (94).

The literature procedure[199] was modified. A solution of $AgNO_3$ (0.54 g, 3.20 mmol) in water (6 mL) was added to a suspension of aldehyde **93** (0.60 g, 3.03 mmol) in 98% ethanol (26 mL). The yellow suspension was stirred rapidly as a solution of 1.0 N NaOH (13.5 mL) was added dropwise over 20 min to form Ag_2O; the dark reaction mixture was stirred vigorously for a further 24 h. Ethanol was removed *in vacuo* and the aqueous residue was filtered through a No. 2 filter paper to remove Ag_2O and metallic silver. The solids were washed with 1.3 N NaOH (2 x 5 mL) and water (5 mL). The combined basic filtrates were extracted with CH_2Cl_2 (2 x 15 mL) to remove the unreacted aldehyde and adjusted to pH 3.5 with 1 : 1 (v/v) 4N HCl/ acetic acid, which produced a white precipitate. The mixture was maintained at -20 °C overnight; the white solid was collected by filtration and vacuum-dried to afford the pure acid **94** (0.36 g, 55%): mp 287-290 °C (lit.[199] mp 271.3-272.3 or 277- 279 °C); IR (KBr) 3650-2400 (br), 1708, 1428, 1244, 1217, 942 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.85 (dd, 1 H, J = 0.6, 4.9 Hz, 6-H), 8.81 (d, 1 H, J = 0.6 Hz, 3-H), 8.57 (d, 1 H, J = 4.9 Hz, 6'-H), 8.26 (br s, 1 H, 3'-H), 7.85 (dd, 1 H, J = 1.6, 5.0 Hz, 5-H), 7.32 (dd, 1 H, J = 0.6, 5.0 Hz, 5'-H), 2.42 (s, 3 H, -CH_3); ^{13}C NMR (50 MHz, DMSO-d_6) δ 166.1, 156.5, 154.3, 150.2, 149.2, 148.1, 139.3, 125.3, 122.8, 121.3, 119.5, 20.6; exact mass 214.0741 (214.0742 calcd for $C_{12}H_{10}N_2O_2$). Anal. Calcd for $C_{12}H_{10}N_2O_2$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.32; H, 4.46; N, 12.94.

(3*S*,4*R*)-3-[(4''-Methyl-2',2''-bipyridine-4'-carbonyl)amino]-4-methyl-2-oxetanone (95).

To a stirred solution of the acid **94** (21.4 mg, 0.10 mmol) and **33** (27.3 mg, 0.10 mmol) in DMF (2 mL) at 0 °C were added diethylphosphoryl cyanide (19.4 mg, 0.11 mmol) and triethylamine (22.3 mg, 0.22 mmol) over 5 min. The resulting deep red solution was stirred at 0 °C for 30 min and then at room temperature for 23 h. The reaction mixture was diluted with EtOAc (20 mL)/benzene (10 mL) and then washed with water (7 mL) and saturated NaCl solution (2 x 8 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was dissolved in a minimum volume of EtOAc and quickly filtered through a small SiO_2 column followed by removing the solvent *in vacuo* to provide **95** (18.0 mg, 61%) as a solid: mp 135-138 °C; IR (KBr) 3438, 3248, 1816, 1650, 1608, 1596, 1533, 1361, 1296, 1120, 1033, 1024, 582 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.84 (m, 2 H, 6'-H and 3'-H), 8.56 (d, 1 H, J = 5.0 Hz, 6''-H), 8.40 (d, 1 H, J = 0.6 Hz, 3''-H), 7.88 (d, 1 H, J = 8.0 Hz, N*H*), 7.80 (dd, 1 H, J = 1.6, 5.0 Hz, 5'-H), 7.25 (dd, 1 H, J = 0.6, 5.0 Hz, 5''-H), 5.85 (dd, 1 H, J = 6.0, 8.0 Hz, 3-H), 5.04 (quint., 1 H, J = 6.0 Hz, 4-H), 2.50 (s, 3 H, ArCH_3), 1.56 (d, 3 H, CH_3CH); exact mass 297.1111 (297.1114 calcd for $C_{16}H_{15}N_3O_3$).

(3*S*,4*R*)-3-(Ethoxycarbonyl)amino-4-methyl-2-oxetanone (96).

The following procedure intended for the preparation of **95** via mixed anhydride formation provided **96** as the major product and **95** as the minor product. A mixture of **94** (21.4 mg, 0.10 mmol) in CH_2Cl_2 (5.0 mL) at -5 °C was treated with triethylamine (10.2 mg, 0.10 mmol) and ethyl chloroformate (11.0 mg, 0.10 mmol). The suspension was stirred for 30 min and **33** (27.3 mg, 0.10 mmol) and pyridine (15.9 mg, 0.20 mmol) were added. The mixture was stirred at -5 °C for 30 min and

then at room temperature overnight. The solvent was removed *in vacuo* and the residue was partitioned between EtOAc (20 mL) and water (20 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give a solid. The ^1H NMR (400 MHz) indicated that this material contained **96** as the major product (87%) and **95** as the minor product (13%). This product mixture was dissolved in a minimum volume of chloroform and quickly filtered through a small SiO_2 column. Concentration of the fractions *in vacuo* gave **96** (7.0 mg, 39%): mp 94-95 °C; IR ($CHCl_3$ cast) 3321, 1853, 1844, 1825, 1692, 1549, 1330, 1273, 1083, 1025 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.45 (m, 2 H, N*H* and 3-H), 4.88 (quint., 1 H, *J* = 6.1 Hz, 4-H), 4.17 (m, 2 H, OCH_2CH_3), 1.47 (d, 3 H, *J* = 6.1 Hz, CH_3CH), 1.27 (t, 3 H, *J* = 7.1 Hz, CH_3CH_2O); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 168.9, 155.5, 74.8, 62.2, 60.4, 15.1, 14.5; exact mass 129.0790 (M^+ - CO_2, 58) (129.0790 calcd for $C_6H_{11}NO_2$); MS (CI, NH_3) 174 (MH^+, 50).

β-Alanine Methyl Ester Hydrochloride (97).

A general procedure[200] to prepare amino acid esters was employed. Dry HCl gas was passed into a suspension of β-alanine (10.0 g, 0.11 mol) in MeOH (150 mL) until a clear solution was obtained. The solution was cooled to 0 °C and more HCl gas was bubbled in to give a saturated solution. After stirring at room temperature for 4 h, the solution was concentrated *in vacuo* to give an off-white solid. The crude product was triturated with ether and then recrystallized from MeOH/ether to afford **97** (13.2 g, 85%) as white crystals: mp 90-95 °C (lit.[217] mp 86 °C); IR (KBr) 3440, 3400, 3020 (br), 1738, 1598, 1570, 1525, 1348, 1222, 1007, 797 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 3.77 (s, 3 H, CO_2CH_3), 3.32 (t, 2 H, *J* = 6.5 Hz, NCH_2), 2.85 (t, 2 H, *J* = 6.5 Hz, CH_2CO); exact mass 103.0633 (M^+ - HCl, 100%) (103.0633 calcd for $C_4H_9NO_2$). Anal. Calcd for $C_4H_{10}ClNO_2$: C, 34.42; H, 7.22; N, 10.03. Found: C, 34.21; H, 7.25; N, 9.84.

***N*-(2,3-Dihydroxy)benzoyl-β-alanine Methyl Ester (98).**

A suspension of 2,3-dihydroxybenzoic acid (0.77 g, 5.0 mmol) in thionyl chloride (3 mL, 41.1 mmol) was heated under reflux for 2 h to give a clear solution. The excess thionyl chloride was removed *in vacuo* and then under high vacuum. The acid chloride residue was dissolved in CH_2Cl_2 (60 mL), cooled to -10 °C, and treated with **97** (0.70 g, 5.0 mmol) and Et_3N (1.4 mL, 10.0 mmol). The reaction mixture was stirred at -10 °C for 30 min and at room temperature overnight. The solvent was removed *in vacuo* and the residue partitioned between EtOAc (100 mL) and water (100 mL). The aqueous phase was extracted with EtOAc (2 x 100 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated to give a dark-grey syrup. Purification by flash chromatography (30-40% EtOAc/hexane) provided **98** (0.66 g, 55%) as a solid: mp 85-87 °C; IR (KBr) 3348, 3500-2300 (br), 1715, 1642, 1591, 1549, 1445, 1370, 1273, 1241, 1196, 1170, 741 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.18 (dd, 1 H, *J* = 1.5, 8.0 Hz, Ar*H*), 6.92 (dd, 1 H, *J* = 1.5, 8.0 Hz, Ar*H*), 6.70 (t, 1 H, *J* = 8.0 Hz, Ar*H*), 3.63 (m, 5 H, NCH_2 and CO_2CH_3), 2.65 (t, 2 H, *J* = 6.7 Hz, CH_2CO); exact mass 239.0794 (39) (239.0794 calcd for $C_{11}H_{13}NO_5$). Anal. Calcd for $C_{11}H_{13}NO_5$: C, 55.23; H, 5.48; N, 5.85. Found: C, 55.18; H, 5.26; N, 5.74.

***N*-(2,3-Dihydroxy)benzoyl-β-alanine (99).**

To a solution of **98** (0.330 g, 1.38 mmol) in MeOH (2.5 mL) at 0 °C was added a 1N NaOH solution (4 mL) dropwise. The reaction mixture was stirred at room temperature for 1.5 h at which point, TLC indicated completion of the reaction. The mixture was cooled to 0 °C and acidified with 1.2 N HCl (3 mL) to pH 5. Methanol was removed *in vacuo* and the remaining aqueous residue was further acidified with 1N HCl to pH 1-2 and then extracted with EtOAc (4 x 5 mL). The combined extracts were dried (Na_2SO_4) and concentrated. The solid residue was purified by flash

chromatography (EtOAc-hexane-HOAc, 4 : 6 : 0.1) to yield **99** (192 mg, 62%) as a white solid: mp 167-170 °C; IR (KBr) 3420, 3330, 3320, 3500-2700 (br), 1704, 1632, 1585, 1540, 1466, 1325, 1266, 1242, 1177, 750 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.19 (dd, 1 H, *J* = 1.5, 8.0 Hz, Ar*H*), 6.92 (dd, 1 H, *J* = 1.5, 8.0 Hz, Ar*H*), 6.70 (t, 1 H, *J* = 8.0 Hz, Ar*H*), 3.62 (t, 2 H, *J* = 6.8 Hz, NCH_2), 2.63 (t, 2 H, *J* = 6.8 Hz, CH_2CO); exact mass 225.0636 (225.0637 calcd for $C_{10}H_{11}NO_5$). Anal. Calcd for $C_{10}H_{11}NO_5$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.49; H, 4.86; N, 6.08.

***N*-[(*tert*-Butoxy)carbonyl]-β-alanine (100).**

A general procedure[201] was employed. To a solution of β-alanine (1.78 g, 20.0 mmol) in dioxane (40 mL), water (20 mL) and 1N NaOH (20 mL) cooled to 0 °C was added di-*tert*-butyl dicarbonate (5.24 g, 24 mmol). The reaction mixture was allowed to warm to room temperature over 20 min and then adjusted to pH 8-9 with 1N NaOH (25 mL). After overnight stirring, the reaction mixture was concentrated to 50 mL and extracted with EtOAc (2 x 20 mL). The aqueous solution, covered with EtOAc (30 mL), was cooled to 0 °C and acidified with 10% $KHSO_4$ to pH 2.5 with stirring. The aqueous phase was extracted with EtOAc (3 x 80 mL), and the combined organic layers were washed with water (3 x 50 mL) and saturated NaCl solution (2 x 50 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give **100** (1.04 g, 28%) as a white solid with the following properties: mp 66-70 °C (lit.[218] mp 73-74 °C); IR (CH_2Cl_2 cast) 3500-2400 (br), 1714, 1520, 1368, 1284, 1251, 1170 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 11.05 (br s, 1 H, CO_2H), 3.40 (m, 2 H, NHCH_2), 2.57 (t, 2 H, *J* = 6.0 Hz, CH_2CO_2H), 1.45 (s, 9 H, C(CH_3)$_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 177.1, 161.0, 36.2, 34,4, 28.2; exact mass 133.0379 (M^+ - C_4H_9, 87) (133.0375 calcd for $C_4H_7NO_4$), CI 190 (MH^+, 45), 379 (M_2H^+). Anal. Calcd for $C_8H_{15}NO_4$: C, 50.78; H, 7.99; N, 7.40. Found: C, 50.51; H, 7.98; N, 6.99.

(3*S*,4*R*)-3-[[3'-(*tert*-Butyloxycarbonylamino)propanoyl]amino]-4-methyl-2-oxetanone (101).

A solution of **100** (37.8 mg, 0.20 mmol) in CH_2Cl_2 (6.0 mL) at -5 °C was treated with triethylamine (20.3 mg, 0.20 mmol) and ethyl chloroformate (22.0 mg, 0.20 mmol). The solution was stirred for 20 min before addition of the tosylate salt **33** (54.6 mg, 0.20 mmol) and triethylamine (40.6 mg, 0.40 mmol). After 30 min at -5 °C, the solution was allowed to warm to 20 °C overnight. The solvent was removed *in vacuo* and the residue was partitioned between EtOAc (40 mL) and water (15 mL). The organic phase was washed with water (10 mL) and saturated NaCl solution (10 mL), dried over Na_2SO_4, and concentrated to afford **101** (35.4 mg, 65%) as a white solid: mp 114-116 °C; IR (KBr disk) 3360, 3342, 3318, 2982, 2937, 1833, 1687, 1660, 1537, 1368, 1290, 1248, 1169, 1020, 840 cm^{-1}; ^1H NMR (200MHz, $CDCl_3$) δ 7.40 (d, 1 H, *J* = 8.1 Hz, N*H*CH), 5.62 (dd, 1 H, *J* = 6.2, 8.1 Hz, 3-H), 5.16 (br s, 1 H, N*H*COC$(CH_3)_3$), 4.89 (quint., 1 H, *J* = 6.2 Hz, 4-H), 3.42 (m, 2 H, NHCH_2), 2.53 (t, 2 H, *J* = 6.0 Hz, COCH_2), 1.43 (m, 12 H, CH_3CH and C(C$H_3)_3$); exact mass 272.1371 (272.1372 calcd for $C_{12}H_{20}N_2O_5$), 228.1475 (M^+ - CO_2) (228.1475 calcd for $C_{11}H_{20}N_2O_3$).

(3*S*,4*R*)-3-(β-Alaninylamino)-4-methyl-2-oxetanone *p*-Toluenesulfonate Salt (102).

A solution of **101** (26.1 mg, 0.096 mmol) and *p*-toluenesulfonic acid (19.0 mg, 0.10 mmol) in trifluoroacetic acid (1 mL) at 0 °C was stirred for 20 min. The solvent was removed under high vacuum, and the residue was triturated with ether to give **102** (33 mg, 99%) as a foam: IR (MeOH/$CHCl_3$ cast) 3400-2600 (br), 1821, 1666, 1638, 1632, 1184, 1123, 1035, 1011, 684, 569 cm^{-1}; ^1H NMR (200 MHz, DMF-d_7) δ 9.18 (d, 1 H, *J* = 8.0 Hz, N*H*CH), 7.65 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 7.15 (d. 2 H, *J* = 8.0 Hz, Ar*H*), 5.70 (dd, 1 H, *J* = 6.0, 8.0 Hz, 3-H), 4.92 (quint., 1 H, *J*

= 6.0 Hz, 4-H), 3.30 (m, 2 H, $CH_2NH_3^+$), 2.86 (t, 2 H, J = 6.0 Hz, $COCH_2$), 2.28 (s, 3 H, CH_3-Ar), 1.39 (d, 3 H, J = 6.0 Hz, CH_3CH); MS (FAB) 345 (MH^+).

(3*S*,4*R*)-3-[[(2',3'-Dihydroxybenzoyl)-β-alaninyl]amino]-4-methyl-2-oxetanone (103).

To a suspension of **102** (31.0 mg, 0.090 mmol) in CH_2Cl_2 (2 mL) at -18 °C were added the acid chloride **63** (25.3 mg, 0.12 mmol) and triethylamine (20.3 mg, 0.20 mmol). After 20 min at -18 °C, the temperature was raised to -10 °C for 1 h and then to room temperature for 10 h. The solvent was removed *in vacuo*, and the residue was partitioned between EtOAc (40 mL) and water (20 mL). The organic phase was washed with water (20 mL) and saturated NaCl solution (10 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give **103** (20.1 mg, 68%) with reasonable purity. An attempt to purify the product by quickly filtering the concentrated EtOAc solution through a small SiO_2 (140 mg) column according to the literature method[170] resulted in a substantial loss of **103** without much improvement of the purity. The final purification was achieved by HPLC (C_{18} reverse phase, isocratic elution with 13% acetonitrile/water) to give **103** as white powder (3.5 mg) with the following properties: IR (KBr) 3440 (br), 1817, 1636, 1541, 1243 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ 8.27 (br d, 1 H, J = 8.0 Hz, N*H*CH), 7.23 (d, 1 H, J = 8.1 Hz, Ar*H*), 6.97 (dd, 1 H, J = 1.2, 8.1 Hz, Ar*H*), 6.72 (t, 1 H, J = 8.1 Hz, Ar*H*), 5.68 (m, 1 H, 3-H), 4.89 (quint., 1 H, J = 6.3 Hz, 4-H), 3.68 (m, 2 H, $NHCH_2$), 2.67 (m, 2 H, $COCH_2$), 1.39 (d, 3 H, J = 6.3 Hz, CH_3CH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 170.0, 169.4, 150.5, 145.8, 142.6, 119.5, 119.1, 117.6, 113.0, 75.1, 59.5, 36.5, 35.4, 15.2; exact mass 308.1006 (308.1008 calcd for $C_{14}H_{16}N_2O_6$), 290.0901 (M^+ - H_2O) (290.0899 calcd for $C_{14}H_{14}N_2O_5$).

Methyl 3,4-Dihydroxybenzoate (104).

A suspension of 3,4-dihydroxybenzoic acid (1.50 g, 10.0 mmol) in thionyl chloride (5 mL) was heated to reflux for 2 h to give a clear yellow solution. Excess thionyl chloride was removed *in vacuo*, and the oily residue was dissolved in CH_2Cl_2 (10 mL) and slowly added to MeOH (10 mL) at 0 °C. After stirring at room temperature for 30 min, the solvent was removed *in vacuo* to give an off-white solid residue. Purification by flash chromatography afforded compound **104** (1.32 g, 78% yield) as a white solid: mp 121-122 °C (lit.[219] mp 134.5 °C); IR ($CHCl_3$ cast) 3468, 3264, 1688, 1611, 1449, 1293, 1268, 1240, 1186, 1165, 1100, 1091, 984, 764 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ and 2 drops of CD_3OD) δ 7.49 (m, 2 H, Ar*H*), 6.88 (d, 1 H, *J* = 4.8 Hz, Ar*H*), 3.87 (s, 3 H, OC*H*$_3$); exact mass 168.0422 (M^+, 51) (168.0423 calcd for $C_8H_8O_4$), 137.0240 (M^+ - OCH_3, 100) (137.0239); Anal. Calcd for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 57.13; H, 4.82.

5-Bromo-2,3-dihydroxybenzoic Acid (105).

A general bromination procedure[203] was adapted. To a stirred suspension of 2,3-dihydroxybenzoic acid (1.54 g, 10.0 mmol) in AcOH (12 mL) was slowly added a solution of Br_2 (1.55 g, 9.69 mmol) in AcOH (3 mL) at room temperature. The mixture became clear within 1 h and the solvent was removed under high vacuum to give a gray solid. The crude product was purified by flash chromatography (1% AcOH/EtOAc) to give pure **105** (2.15 g, 92 %): mp 202-205 °C (lit.[220] mp 215 °C); IR (CH_2Cl_2 cast) 3700-2400 (br), 1676, 1467, 1298, 1265, 1216, 1161 cm^{-1}; ^1H NMR (200 MHz, DMF-d_7) δ 9.75 (br s, 1 H, COO*H*), 7.50 (d, 1 H, *J* = 3.0 Hz, Ar*H*), 7.19 (d, 1 H, *J* = 3.0 Hz, Ar*H*); exact mass 231.9372 (M^+, ^{79}Br) (231.9372 calcd for $C_7H_5O_4{}^{79}Br$), 233.9354 (M^+, ^{81}Br) (233.9351 calcd for $C_7H_5O_4{}^{81}Br$). Anal. Calcd for $C_7H_5O_4Br$: C, 36.08; H, 2.16. Found: C, 36.28; H, 2.33.

***N*-Methyl 5-Bromo-2,3-dihydroxybenzamide (106).**

A suspension of **105** (1.76 g, 7.55 mmol) in thionyl chloride (5 mL) was heated under reflux for 2 h and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. CH_3NH_2 gas was passed into the solution for 5 min, after which TLC showed the reaction to be complete. After removal of the solvent *in vacuo*, the residue was purified by flash chromatography to give **106** (0.89 g, 48 %) as colorless crystals: mp 132-133 °C; IR (CH_2Cl_2 cast) 3466, 3357, 3500-2400 (br), 1636, 1596, 1551, 1466, 1320, 1266, 1236, 1175, 791 cm^{-1}; ^1H NMR (200 MHz, $(CD_3)_2CO$) δ 8.19 (br s, 1 H, N*H*CH_3), 7.37 (d, 1H, *J* = 2.2 Hz, Ar*H*), 7.08 (d, 1H, *J* = 2.2 Hz, Ar*H*), 2.92 (d, 3H, *J* = 4.6 Hz, NHC*H*$_3$); exact mass 244.9684 (78) (244.9688 calcd for $C_8H_8{}^{79}BrNO_3$), 246.9665 (78) (246.9667 calcd for $C_8H_8{}^{81}BrNO_3$). Anal. Calcd for $C_8H_8BrNO_3$: C, 39.05; H, 3.28; N, 5.69; Br, 32.47. Found: C, 39.07; H, 3.06; N, 5.69; Br, 32.59.

Benzyl 2,3-Dibenzyloxy-5-bromobenzoate (107).

To a stirred suspension of 5-bromo-2,3-dihydroxybenzoic acid (**105**) (0.233 g, 1.00 mmol) and KOH (0.224 g, 4.00 mmol) in DMSO (3 mL) was added benzyl bromide (0.5 mL, 4 mmol). After 1.5 h, the KOH particles had disappeared and the whole mixture became a slurry. The reaction was stopped by adding water (20 mL), and the mixture was extracted with ether (3 x 20 mL). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. The residue was applied to a flash chromatography column (5-10% EtOAc-hexane) to afford compound **107** (0.35 g, 70%) as a white solid: mp 79-81 °C; IR ($CHCl_3$ cast) 1728, 1569, 1472, 1454, 1373, 1308, 1258, 1043, 1027, 736, 696 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.50 (d, 1 H, *J* = 2.4 Hz, Ar*H*), 7.37 (m, 15 H, Ar*H*), 7.25 (d, 1 H, *J* = 2.4 Hz, Ar*H*), 5.29 (s, 2 H, CO_2CH_2Ph), 5.09 (s, 2 H, ArOCH_2Ph), 5.01 (s, 2 H, ArOCH_2Ph); exact mass 504.0760 (504.0760 calcd for $C_{28}H_{23}{}^{81}BrO_4$), 502.0782 (502.0780 calcd for

$C_{28}H_{23}{}^{79}BrO_4$), MS (CI, NH_3) 505 (MH^+, ^{81}Br, 55)/503 (MH^+, ^{79}Br, 55). Anal. Calcd for $C_{28}H_{23}BrO_4$: C, 66.81; H, 4.61; Br, 15.87. Found: C, 66.80; H, 4.63; Br, 15.57.

_N_-Methyl-2-hydroxy-3-benzyloxy-5-bromobenzamide (108).

A suspension of **107** (5.47 g, 10.9 mmol) in EtOH (20 ml) was treated with a solution of methylamine (10.5 g, 0.338 mol) in EtOH (50 mL). After 3 h at room temperature, THF (90 mL) was added to give a clear solution which was stirred for a further 48 h. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (20-40% EtOAc-hexane) to give **108** (2.76 g, 75%) as a white solid: mp 158-159 °C; IR ($CHCl_3$ cast) 3392, 1647, 1601, 1583, 1463, 1359, 1331, 1238, 1002, 860, 750 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.41 (m, 5 H, $ArOCH_2C_6H_5$), 7.26 (d, 1 H, J = 2.4 Hz, Ar*H* (6-H)), 7.08 (d, 1 H, J = 2.4 Hz, Ar*H* (4-H)), 6.75 (d, 1 H, J = 5.0 Hz, $NHCH_3$), 5.12 (s, 2 H, OCH_2Ph), 2.97 (d, 3 H, J = 5.0 Hz, $NHCH_3$); exact mass 337.0131 (6.8) (337.0136 calcd for $C_{15}H_{14}{}^{81}BrNO_3$), 335.0154 (7.0) (335.0157 calcd for $C_{15}H_{14}{}^{79}BrNO_3$). Anal. Calcd for $C_{15}H_{14}BrNO_3$: C, 53.59; H, 4.20; N, 4.17; Br, 23.77. Found: C, 53.51; H, 4.12; N, 4.18; Br, 23.84.

This reaction was also repeated using THF as the only solvent and the same major product was obtained except that an additional 48 h was required for the reaction to reach completion.

2,3-[(Diphenylmethylene)dioxy]benzene (109).

The literature procedure[205] was modified. A suspension of catechol (5.50 g, 50.0 mmol) and dichlorodiphenylmethylene (12.33 g, 52.0 mmol) was heated at 170 °C for 5 min. The reaction mixture was cooled to room temperature, and the resulting solid was recrystallized twice from ethanol to give **109** (6.38 g, 46%) as white

crystals: mp 88-90 °C (lit.[205] mp 87-89 °C); IR ($CHCl_3$ cast) 2957, 1488, 1449, 1260, 1241, 1214, 1046, 1019, 809, 740, 699 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.62 (m, 4 H, Ar*H*), 7.37 (m, 6 H, Ar*H*), 6.88 (m, 4 H, Ar*H*); exact mass 274.0990 (56) (274.0994 calcd for $C_{19}H_{14}O_2$), 197.0605 (100) ($M^+ - C_6H_5$) (197.0603 calcd for $C_{13}H_9O_2$). Anal. Calcd for $C_{19}H_{14}O_2$: C, 83.19; H, 5.14. Found: C, 82.81; H, 5.11.

2,3-[(Diphenylmethylene)dioxy]benzoic Acid (110).

The literature procedure[205] was modified. A solution of **109** (2.74 g, 10.0 mmol) in THF (30 mL) was treated with *n*-butyllithium (1.53M in hexane, 7.84 mL, 12.0 mmol) at -20 °C and then stirred for 2 h at room temperature. The reaction solution was poured onto solid carbon dioxide (60 g) in dry ether (30 mL) with shaking. After reaching room temperature, the reaction mixture was partitioned between water (50 mL) and ether (50 mL). The alkaline aqueous layer was repeatedly extracted with ether (3 x 25 mL) while the pH was continuously adjusted with 1N HCl to 8.0-8.5. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to afford **110** (1.15 g, 36%): mp 165-170 °C (lit.[205] mp 188-189 °C); IR (KBr) 3500-2200 (br), 1692, 1634, 1480, 1465, 1449, 1424, 1310, 1265, 1248, 1210, 1057, 1020, 746, 696 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.62 (m, 4 H, Ar*H*), 7.46 (dd, 1H, *J* = 1.5, 8.0 Hz, Ar*H*), 7.36 (m, 6 H, Ar*H*), 7.06 (dd, 1 H, *J* = 1.5, 8.0 Hz, Ar*H*), 6.88 (t, 1 H, *J* = 8 Hz, Ar*H*); exact mass 318.0891 (61) (318.0892 calcd for $C_{20}H_{14}O_4$), 241.0500 ($M^+ - C_6H_5$, 100) (241.0501 calcd for $C_{14}H_9O_4$). Anal. Calcd for $C_{20}H_{14}O_4$: C, 75.46; H, 4.43. Found: C, 75.76; H, 4.46.

3,4-Dioxosulfinylbenzoyl Chloride (111).*

A mixture of 3,4-dihydroxybenzoic acid (2.00 g, 13.0 mmol) and thionyl chloride (7.0 mL, 96.0 mmol) was heated to reflux for 5 h to give a clear, brownish solution. Excess thionyl chloride was removed *in vacuo* and the residue was distilled to provide **111** (2.48 g, 87%) as a clear liquid (bp 109-110 °C at 1.0 mm Hg): IR ($CHCl_3$ cast) 1785, 1745, 1608, 1483, 1428, 1260, 1233, 1195, 1092, 945, 814, 801, 708 cm^{-1}; ^1H NMR (400 MHz, CD_3Cl) δ 8.04 (d, 1 H, *J* = 8.5 Hz, Ar*H*), 7.96 (s, 1 H, Ar*H*), 7.31 (d, 1 H, *J* = 8.5 Hz, Ar*H*); exact mass 219.9413 (M^+, ^{37}Cl, 6) (219.9411 calcd for $C_7H_3O_4{}^{37}ClS$) 217.9440 (M^+, ^{35}Cl, 17) (217.9440 calcd for $C_7H_3O_4{}^{35}ClS$).

(3*S*,4*R*)-3-(3,4-Dihydroxybenzoyl)amino-4-methyl-2-oxetanone (112).*

A mixture of **33** (50.0 mg, 0.18 mmol) in CH_2Cl_2 (1.5 mL) was cooled to -10 °C and treated with the acid chloride **111** (80.0 mg, 0.37 mmol) and pyridine (30 μL, 0.37 mmol). The reaction mixture was stirred at -10 °C for 30 min and then at room temperature for 20 h. The mixture was partitioned between water (10 mL) and EtOAc (10 mL), and the organic phase was washed with water (10 mL), dried over $MgSO_4$, and concentrated. The crude product was applied to a small flash chromatography column and eluted with EtOAc to give **112** (53 mg, 38%): ^1H NMR (200 MHz, $(CD_3)_2CO$) δ 8.90 (d, 1 H, *J* = 8.0 Hz, N*H*), 7.47 (d, 1 H, *J* = 3.0 Hz, Ar*H*), 7.38 (dd, 1 H, *J* = 3.0, 8.0 Hz, Ar*H*), 6.88 (d, 1 H, *J* = 8.0 Hz, Ar*H*), 5.84 (dd, 1 H, *J* = 6.0, 8.0 Hz, 3-H), 4.95 (quint., 1 H, *J* = 6 Hz, 4-H), 1.49 (d, 3 H, *J* = 6.0 Hz, C*H*$_3$CH); exact mass 237.0638 (M^+, 5) (237.0637 calcd for $C_{11}H_{11}NO_5$), 219.0532 ($M^+ - H_2O$, 13) (219.0532 calcd for $C_{11}H_9NO_4$).

Ethyl (4-Pyridylthio)acetate (113).[221]

4-Mercaptopyridine (1.55 g, 12.5 mmol) was added to a stirred suspension of NaOH (0.56 g, 14.0 mmol) in EtOH (98%, 50 mL). After 5 min at room temperature, ethyl bromoacetate (2.20 g, 12.5 mmol) was added, and the mixture was stirred overnight. The solvent was removed *in vacuo*, and the residue was partitioned between water (100 mL) and EtOAc (100 mL). The aqueous layer was extracted with EtOAc (2 x 100 mL) and the combined extracts were dried over Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography (EtOAc) to give **113** (2.47 g, quantitative) as an oil: IR (neat) 2981, 1734, 1575, 1541, 1483, 1408, 1296, 1270, 1221, 1179, 1155, 1027, 802, 704 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.42 (br d, 2 H, *J* = 4.9 Hz, Ar*H*), 7.17 (dd, 2 H, *J* = 1.5, 4.9 Hz, Ar*H*), 4.21 (q, 2 H, *J* = 8.0 Hz, OCH_2CH_3), 3.75 (s, 2 H, $ArSCH_2$), 1.26 (t, 3 H, *J* = 8.0 Hz, OCH_2CH_3); exact mass 197.0512 (77) (197.0511 calcd for $C_9H_{11}NO_2S$). Anal. Calcd for $C_9H_{11}NO_2S$: C, 54.80; H, 5.62; N, 7.10; S, 16.25. Found: C, 54.56; H, 5.57; N, 7.12, S, 16.21.

(4-Pyridylthio)acetic Acid (114).

A mixture of **113** (0.32 g, 1.62 mmol) in 2.5 N NaOH (5 mL) was heated at reflux for 40 min. The resulting yellow solution was cooled to room temperature, acidified to pH 1-2 with concentrated HCl, and concentrated *in vacuo*. The off-white solid residue was purified on an ion-exchange column (AG 50W-X8, H^+ form) which was eluted with water followed by 1M NH_4OH. The combined fractions were first concentrated *in vacuo* to remove ammonia and then lyophilized to afford **114** (0.25 g, 91%) as a white powder: mp 255-258 °C (dec.) (lit.[222] mp 251-255 °C dec.); IR (KBr) 3431 (br), 1702, 1656, 1630, 1620, 1482, 1198, 1054, 816 cm^{-1}; ^1H NMR (200 MHz, D_2O + 1 drop of DCl) δ 8.44 (d, 2 H, *J* = 7.1 Hz, Ar*H*), 7.81 (d, 2 H, *J* = 7.1 Hz, Ar*H*), 4.20 (s, 2 H, $ArSCH_2$); exact mass 169.0196 (100) (169.0197 calcd

for $C_7H_7NO_2S$). Anal. Calcd for $C_7H_7NO_2S$: C, 49.69; H, 4.17; N, 8.28; S, 18.95. Found: C, 49.57; H, 4.05; N, 8.30; S, 19.06.

(3*S*,4*R*)-3-[(4-Pyridylthioacetyl)amino]-4-methyl-2-oxetanone (115). Method **A**. To a stirred suspension of acid **114** (16.9 mg, 0.10 mmol) and **33** (27.3 mg, 0.10 mmol) in DMF (2 mL) at 0 °C were added diethylphosphoryl cyanide (19.4 mg, 0.11 mmol) and triethylamine (25.3 mg, 0.25 mmol) over 5 min. The mixture became clear at 0 °C within 1 h and was then allowed to warm to room temperature over 20 h. The reaction mixture was diluted with EtOAc (20 mL)/benzene (10 mL), and washed with water (8 mL) and saturated NaCl solution (2 x 8 mL). The organic solution was dried over Na_2SO_4 and concentrated *in vacuo* to give **115** (28.5 mg, 96%) as an oil with good purity. This product was however unstable; it polymerized to a solid in several hours. IR ($CHCl_3$) 1830, 1665 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 8.35 (dd, 2 H, *J* = 1.5, 4.5 Hz, Ar*H*), 7.39 (dd, 2 H, *J* = 1.5, 4.5 Hz, Ar*H*), 5.54 (d, 1 H, *J* = 6.0 Hz, 3-H), 4.88 (quint., 1 H, *J* = 6.0 Hz, 4-H), 3.89 (s, 2H, ArSCH_2), 1.45 (d, 3 H, *J* = 6.0 Hz, CH_3CH).

Method **B**. Sodium 4-pyridylsulfide was prepared by reaction of 4-mercapto-pyridine (10 mg, 0.090 mmol) in DMF (3 mL) with NaH (2.5 mg, 0.10 mmol) for 10 min at room temperature. This solution was immediately added dropwise to a solution of **118** (20.1 mg, 0.090 mmol) in CH_2Cl_2 (2 mL) with vigorous stirring. After an additional 10 min, the reaction mixture was partitioned between EtOAc (20 mL)/benzene (10 mL) and water (10 mL). The organic layer was washed with water (10 mL) and saturated NaCl solution (10 mL), and was then dried over Na_2SO_4. Evaporation of the solvent yielded **115** (22.0 mg, 97 %) with an identical ^1H NMR spectrum (200 MHz) to that prepared by Method A. This product, as described above, was unstable and quickly polymerized to a solid.

Ethyl (6-Purinylthio)acetate (116).

To a suspension of NaOH (0.5 g, 12.5 mmol) in EtOH (98%, 50 mL) were added 6-mercaptopurine monohydrate (2.15 g, 12.5 mmol) and a solution of ethyl bromoacetate (2.20 g, 12.5 mmol) in ethanol (10 mL). The mixture became clear over one hour, after which TLC indicated the reaction to be complete. The solvent was removed *in vacuo* and the off-white solid residue was suspended in water (50 ml) and extracted with EtOAc (4 x 50 ml). The combined organic extracts were dried (Na_2SO_4), concentrated *in vacuo*, and purified by flash chromatography (EtOAc) to afford **116** (2.85 g, 96%) as a white solid: mp 122-124 °C (lit.[223] mp 128-130 °C); IR (KBr) 3420, 1734, 1572, 1385, 1307, 1237, 1181, 1160, 949, 642 cm^{-1}; ^1H NMR (200 MHz, $(CD_3)_2CO$) δ 8.62 (s, 1 H, Ar*H*), 8.39 (s, 1 H, Ar*H*), 4.23 (s, 2 H, $ArSCH_2$), 4.16 (q, 2 H, J = 7.2 Hz, $CO_2CH_2CH_3$), 1.22 (t, 3 H, J = 7.2 Hz, $CO_2CH_2CH_3$); exact mass 238.0525 (238.0524 calcd for $C_9H_{10}N_4O_2S$). Anal. Calcd for $C_9H_{10}N_4O_2S$: C, 45.37; H, 4.23; N, 23.51; S, 13.46. Found: C, 45.10; H, 4.19; N, 23.22; S, 13.11.

(6-Purinylthio)acetic Acid (117).

A solution of **116** (0.50 g, 2.10 mmol) in 2.5 N NaOH (5 mL) was heated at reflux for 1 h, after which TLC indicated the completion of the reaction. The solution was cooled to room temperature, diluted to 50 mL with water, and extracted with EtOAc (2 x 50 mL). The remaining aqueous solution was acidified with concentrated HCl to pH 1-2, and then concentrated *in vacuo*. The off-white solid residue was suspended in 20 mL of boiling water, first cooled to room temperature and then to -5 °C. The white crystals were collected by filtration, washed with ether, and dried under high vacuum to yield **117** (359 mg, 81%). mp 245-250 °C dec. (lit.[224] mp 250-252 °C); IR (KBr) 3600-2200 (br), 1711, 1596, 1573, 1439, 1420, 1395, 1322, 1309, 1239, 1194, 1002, 949, 859 cm^{-1}; ^1H NMR (200 MHz, D_2O + DCl) δ 8.72 (s, 1 H,

Ar*H*), 8.38 (s, 1 H, Ar*H*), 3.80 (s, 2 H, ArSC*H*$_2$); exact mass 210.0209 (210.0211 calcd for $C_7H_6N_4O_2S$). Anal. Calcd for $C_7H_6N_4O_2S$: C, 40.00; H, 2.88; N, 26.65; S, 15.25. Found: C, 39.94; H, 2.75; N, 26.37; S, 15.15.

(3*S*, 4*R*)-3-(Bromoacetylamino)-4-methyl-2-oxetanone (118).

A mixture of **33** (47.0 mg, 0.17 mmol) in CH_2Cl_2 (7 mL) at -10 °C was treated with pyridine (33.3 mg, 0.42 mmol) and bromoacetyl chloride (33.1 mg, 0.20 mmol). After stirring at -10 °C for 1 h, the mixture was allowed to warm to 0 °C over 2 h. It was then stirred at 20 °C for 8 h. The solvent was removed *in vacuo* to give an oily residue that was then partitioned between EtOAc (60 mL) and water (50 mL). The aqueous layer was further extracted with EtOAc (30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to afford **118** (35.8 mg, 95%) as an oil that crystallized upon standing. This compound exists as a mixture of two conformers in a 4:1 (**A**/**B**) ratio as indicated by ^{1}H and ^{13}C NMR: mp 107-110 °C; IR ($CHCl_3$ cast), 3280 (br), 1850, 1810, 1675, 1664, 1541, 1290, 1128 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39 (br, 0.8 H, N*H*, **A**), 7.31(br, 0.2 H, N*H*, **B**), 5.63 (m, 1 H, C*H*NH), 4.94 (quint., 1 H, *J* = 6.3 Hz, CH_3CH), 4.13 (s, 1.6 H, BrC*H*$_2$CO, **A**), 3.94, 3.92 (2 x s, 0.4 H, BrC*H*$_2$CO, **B**), 1.48 (d, 3 H, *J* = 6.3 Hz, C*H*$_3$CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2 (C-2), 166.4 (*C*OCH$_2$Br, **A**), 166.1 (*C*OCH$_2$Br, **B**), 76.6 (C-4, **B**), 76.4 (C-4, **A**), 59.1 (C-3, **B**), 58.8 (C-3, **A**), 42.1 (Br*C*H$_2$, **A**), 27.8 (Br*C*H$_2$, **B**), 14.9 (*C*H$_3$); exact mass 176.9789 (M^+ - CO_2) (176.9790 calcd for $C_5H_8{}^{79}BrNO$), 142.0504 (M^+ - Br) (142.0504 calcd for $C_6H_8NO_3$).

(3*S*,4*R*)-3-(6-Purinylthioacetylamino)-4-methyl-2-oxetanone (119).

A solution of **118** (14.7 mg, 0.066 mmol) and 6-mercaptopurine monohydrate (11.2 mg, 0.073 mmol) in DMF (1 mL) was treated with triethylamine (8.71 mg, 0.086 mmol). The reaction mixture was stirred overnight, the solvent was removed *in*

vacuo, and the residue was partitioned between EtOAc (20 mL) and water (20 mL). The organic phase was dried over Na_2SO_4 and concentrated to give **119** (12.7 mg, 66%) as a solid with the following properties: IR (KBr) 3440, 3700-2400 (br), 1821, 1665, 1572, 1547, 1384, 1325 cm^{-1}; ^1H NMR (200 MHz, $(CD_3)_2CO$) δ 8.68 (s, 1 H, Ar*H*), 8.58 (br s, 1 H, N*H*), 8.40 (s, 1 H, Ar*H*), 5.66 (m, 1 H, 3-H), 4.89 (quint., 1 H, *J* = 6.3 Hz, 4-H), 4.21, 4.19 (2 x s, 2 H, ArSCH_2CO), 1.37 (d, 3 H, *J* = 6.3 Hz, CH_3CH); exact mass 275.0471 (M^+ - H_2O) (275.0477 calcd for $C_{11}H_9N_5O_2S$).

L-α-(*N*-Benzyloxycarbonyl)aminoadipic Acid (120).

The literature procedure[209,210] was adapted. Benzyloxycarbonyl chloride (1.02 mL, 1.22 g, 6.80 mmol) was added dropwise over 10 min to a vigorously stirred suspension of L-α-aminoadipic acid (1.00 g, 6.20 mmol) and $NaHCO_3$ (1.82 g, 21.7 mmol) in water (14 mL). After stirring at room temperature for 22 h, the reaction mixture was extracted with ether (10 mL) and the aqueous layer was acidified at 0 °C to pH 1-2 with concentrated HCl. The resulting mixture was extracted with EtOAc (3 x 10 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give a white solid (1.61 g). This material was dissolved in a minimum amount of hot EtOAc and then sufficient petroleum ether was added just to begin precipitation. More petroleum ether (10 mL) was added and the mixture was left at 4 °C overnight. The solid residue was collected by filtration and was further purified by recrystallization from water to afford **120** (1.26 g, 69%) as white crystals: mp 124-128 °C (lit.[225] mp 131-133 °C); IR (KBr) 3450, 3307, 2300-3600 (br), 1706, 1696, 1536, 1280 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.32 (m, 5 H, Ar*H*), 5.09 (s, 2 H, OCH_2Ph), 4.14 (m, 1 H, NC*H*CO_2H), 2.31 (m, 2 H, HO_2CCH_2), 1.55-1.95 (m, 4 H, $HO_2CCH_2CH_2CH_2$); exact mass 295.1058 (295.1056 calcd for $C_{14}H_{17}NO_6$). Anal. Calcd for $C_{14}H_{17}NO_6$: C, 56.95; H, 5.80; N, 4.74. Found: C, 56.53; H, 5.69; N, 4.74.

(*S*)-3-(Benzyloxycarbonyl)-4-(3-carboxypropyl)-5-oxazolidinone (121).

The literature procedure[209,210] was applied. A mixture of **120** (1.00 g, 3.39 mmol), paraformaldehyde (0.203 g, 6.78 mmol), powdered 4 Å molecular sieves (1.48 g), and *p*-toluenesulfonic acid (38.0 mg, 0.20 mmol) in benzene (20 mL) was heated to reflux for 24 h and filtered hot. The filtrate was washed with water (2 x 8 mL) and extracted with 5% $NaHCO_3$ solution (2 x 10 mL). The combined extracts were acidified with 6 N HCl to pH 1-2 and extracted with EtOAc (3 x 10 mL). The organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give **121** as a colorless oil (100 mg, 10%). IR ($CHCl_3$ cast) 3500-2400 (br), 1802, 1710, 1418, 1358 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 10.05 (br s, 1 H, CO_2H), 7.36 (s, 5 H, Ar*H*), 5.53 (m, 1 H, NC*H*HO), 5.20 (m, 3 H, NCH*H*O and OCH_2Ph), 4.33 (t, 1 H, *J* = 5.2 Hz, NC*H*CO), 2.37 (m, 2 H, HO_2CCH_2), 2.14-1.58 (m, 4 H, $HO_2CCH_2CH_2CH_2$); exact mass 307.1053 (307.1056 calcd for $C_{15}H_{17}NO_6$).

(3*S*,4*R*,4''*S*)-3-[4'-[3''-(Benzyloxycarbonyl)-5''-oxazolidinon-4''-yl]-butanoylamino]-4-methyl-2-oxetanone (122).

A solution of **121** (43.4 mg, 0.15 mmol) in CH_2Cl_2 (5.0 mL) at -5 °C was treated with triethylamine (15.2 mg, 0.15 mmol) and ethyl chloroformate (16.5 mg, 0.15 mmol). The solution was stirred for 30 min, and then **33** (41.0 mg, 0.15 mmol) and pyridine (23.8 mg, 0.30 mmol) were added. After an additional 30 min at -5 °C, the solution was allowed to warm to 20 °C overnight. The solvent was removed *in vacuo* and the residue was partitioned between EtOAc (40 mL) and water (40 mL). The organic phase was dried (Na_2SO_4), and concentrated *in vacuo* to afford **122** (51.7 mg, 90%) as an oil: IR ($CHCl_3$ cast) 3330, 2960, 2930, 1830, 1808, 1720, 1536, 1457, 1418, 1359, 1245, 1125, 1050, 1021, 754 cm^{-1}; ^1H NMR (200MHz, $CDCl_3$) δ 7.37 (s, 5 H, Ar*H*), 6.99 (br s, 1 H, N*H*), 5.58 (m, 2 H, 3-H and 2"-H), 5.21 (m, 3 H, 2"-H and ArCH_2), 4.88 (quint., 1 H, *J* = 6.2 Hz, 4-H), 4.35 (m, 1 H, 4"-H),

2.34 (m, 2 H, 2'-H), 1.60-2.15 (m, 4 H, 3'-H and 4'-H), 1.42 (d, 3 H, J = 6.2 Hz, CH_3CH); exact mass 390.1444 (390.1427 calcd for $C_{19}H_{22}N_2O_7$), 372.1327 ($M^+ - H_2O$) (372.1322 calcd for $C_{19}H_{20}N_2O_6$).

Biological Assays of β-Lactone Compounds

The biological tests were done by Dr. Miloslav Sailer of our research group using agar diffusion method.[226,227] Dr. Chris Lowe collaborated in preparing the samples for the assays. The synthetic α-amino β-lactone derivatives were divided into three groups based on their structures for the biological activity tests.

Group 1. Solutions of **2**, **65**, **66** and **67** in acetonitrile were prepared with an initial concentration of 200 μg/mL. Each of the solutions was repeatedly diluted two-fold to give concentrations of 100, 50, 25, 12.5, and 6.2 μg/mL. The organisms used for the assay were *Staphylococcus aureus* ATCC 25923 and 13565, *Escherichia coli* ATCC 11229 and 11775, and *Pseudomonas aeruginosa* ATCC 15442. One drop of each solution was evenly applied to a growing plate of the organisms, and the solvent acetonitrile was also spotted for a control test. The inhibition zone was observed, and the results were recorded after 24 h.

Group 2. Solutions of the following 10 L-threonine β-lactone compounds, **1**, **23**, **33**, **88**, **95**, **96**, **103**, **112**, **118**, and **119**, in a mixed solvent of DMSO/water (20 : 80) were prepared with an initial concentration of 500 μg/mL. Each of the solutions was repeatedly diluted two-fold to give concentrations of 250, 125, and 62 μg/mL. Five organisms: *Staphylococcus aureus* ATCC 6538, *Streptococcus faecalis* ATCC 7080, *Serratia marcescens* ATCC 13380, *Klebsiella pneumoniae* ATCC 11296, and

Proteus vulgaris ATCC 13315 were used for the assay. One drop of each sample was applied evenly to a growing plate of the organisms, and the solvent was also spotted for a control test. The inhibition zone was observed, and the results were recorded after 24 h.

Group 3. Solutions of the following five (*N*-*o*-nitrophenyl)sulfenyl α-amino β-lactone compounds, **23**, **32**, **61**, **86**, and **87**, *o*-nitrothiophenol (**37**), and di-(*o*-nitrophenyl) disulfide (**36**) in a mixed solvent of DMSO/water (50 : 50) were prepared with an initial concentration of 100 μg/mL. Each of the solutions was repeatedly diluted two-fold to give concentrations of 50, 25, 12.5, 6.2, 3.1, and 1.5 μg/mL. The organism *Staphylococcus aureus* ATCC 6538 was used for the assay. One drop of each sample was applied evenly onto a growing plate of the organisms. The pure solvent was also spotted for a control test. The inhibition zone was observed, and the results were recorded after 24 h.

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